5, Vol. 51, 1985

PIBSBB 51 (5) 537-646 (1985)

ISSN 0073-6600

Biological
Sciences
Part B

PROCEEDINGS OF THE INDIAN NATIONAL SCIENCE ACADEMY

INDIAN NATIONAL SCIENCE ACADEMY
NEW DELHI

Proceedings of the Indian National Science Academy Part B

Annual Subscription Rates

(*Including postage*)

inland: Rs. 150.00

foreign: $ 50.00

Single Issue

inland: Rs. 30.00

foreign: $ 10.00

Editorial Office

The Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 110002, India

Proceedings of the Indian National Science Academy Part B, is published in alternate months, i.e., February, April, June, August, October and December

Information to Contributors

Purpose and Scope

Part B of the Proceedings is devoted to papers embodying results of original research in any branch of Life Sciences. The major emphasis of the journal will be on publication of studies especially those with Inter-disciplinary implications relating to Physiology, Cell Biology, Cytogenetics, Genetics, Ecology, Biochemistry, Microbiology, Pathology, Endocrinology, Physiology of Reproduction, etc., relating to plants and animals.

Papers presented at the Academy's symposia, special Academy lectures, original review articles relating to research and concepts fundamental to Life Sciences are also published in the Proceedings.

roc. Indian natn. Sci. Acad. B51 No. 5 pp 537-542 (1985)

Organophosphate-Induced Biochemical Changes in the Brain, Liver and Ovary of the Fish, *Channa punctatus* (Bloch)

RAJ NARAYAN RAM and A G SATHYANESAN
Department of Zoology, Banaras Hindu University, Varanasi 221005

(Received 8 August 1985)

In the organophosphate pesticide (OPP) cythion-treated *C. punctatus* the brain protein content significantly decreased on the 15th and 30th days, whereas in the ovary and liver such reduction was seen on the 15th and 60th days, respectively. This decrease had direct correlation with exposure time for brain and ovary but inverse for liver. Total RNA level was lowered significantly in the brain on the 15th and 60th days; in liver after 30th to 60th days and in ovary only on the 60th day after treatment. A significant reduction in DNA content was also recorded in brain on the 60th day; liver from 45th to 60th days and ovary from 30th to 60th days after cythion exposure. RNA and DNA depletion has an inverse correlation with exposure time in all the tissues. These results suggest that cythion is capable of impairing the physiological functions of the organs studied, in this species.

Key Words: Cythion, Protein, RNA, DNA, *Channa punctatus*

Introduction

Pesticides and their residues washed into the water system adversely affect the growth and survival of fishes (Holden 1973, Johnson 1973). As chlorinated hydrocarbons persist in the environment for a long time, there is an increase in the use of organo-phosphate pesticides (OPPs) in agriculture, forestry and public health. Histopathological effects of OPPs on different organs of fishes have been studied by several investigators (Anees 1978, Dubale & Shah 1979, Mandal & Kulshresthra 1980, Lakota et al. 1982). In fish, apart from their well known cholinesterase (ChE) inhibitory effect, OPPs modify the activity of several other metabolic enzymes (Dalela et al. 1978, Mukhopadhyay & Dehadrai, 1980a,b, Sastry & Sharma 1980, Joshi & Desai 1981, Dubale & Awasthi 1984, Natarajan 1984), proteins (Mukhopadhyay & Dehadrai 1980 a, b, Oishi et al. 1980, Choudhari & Chakrabarti 1983, Dubale & Awasthi 1984) and free amino acids (Natarajan 1983). The mutagenic effects of OPPs have been reviewed by Wild (1975). However, long term effects of sublethal dose of OPPs on different aspects of the physiology of fishes are little known. In the present communication the changes induced

by cythion which is 50% malathion (O, O-Dimethyl Phosphorodithioate of Diethylmercapto Succinate), a commonly used organophosphate pesticide on protein, RNA and DNA contents of the brain, liver and ovary of the fish *C. punctatus* are reported.

Materials and Methods

Over forty adult female *C. punctatus* weighing 40–45 g and having a length of 10–12 cm were bought from the local fish market at Varanasi. They were kept in 40 L glass aquaria containing chlorine free water of *p*H 7.2, hardness 154 ppm (as $CaCO_3$), alkalinity 68 ppm (as $CaCO_3$), dissolved oxygen 7.2 ppm, and conductivity 0.56 mMhos, and acclimated to laboratory conditions for 10 days prior to using in the experiment. Specimens were divided into two equal groups. Animals in group-I were exposed to a sublethal dose of 4.0 ppm commercial cythion while those in group-II served as controls. The highest pesticide concentration in which no mortality was observed during 96 hrs of exposure, but fish appeared to be in stress, has been expressed as 'sublethal dose'. The pesticide used in this investigation was manufactured by Northern Minerals Pvt. Ltd., Haryana and marketed by Dhanuka Marketing Company, Hyderabad, India. Aquaria water was changed every alternate day after feeding the fish with goat liver and pesticide concentration was renewed. At intervals of 15, 30, 45 and 60 days, five fish from each group were decapitated and tissues were frozen and processed for the assays. The technique of Lowry et al. (1951) and the method of Munro and Fleck (1966) were used for assessing total protein, RNA and DNA levels, respectively. The results are given in terms of the pesticide's commercial formulation and not active ingredient of pure pesticide, because only former is used in agriculture.

Student's t-test was used for analyzing the data. Correlation coefficient (r) and regression line equation ($Y=a+bX$) were calculated using the formulae of Bruning and Kintz (1977).

Results

Protein: In *C. punctatus* exposed to cythion, the brain protein content decreased significantly initially at 15th ($P<0.01$) and 30th days ($P<0.05$), and returned to normal on the 45th day. Decrease in liver protein ($P<0.05$) level occurred only on the 60th day of exposure. Ovarian protein was reduced ($P<0.05$) on the 15th day of treatment and it returned to pretreatment level on 30th day. Reduction in protein content correlated directly with exposure time in brain ($r=+0.859$, $P<0.01$), ovary ($r=+0.095$) and inversely in liver ($r=-0.666$) (table 1).

RNA: Brain RNA content was significantly depleted ($P<0.05$) on the 15th day of exposure, returned to normal level between 30th to 45th days and again reduced ($P<0.01$) on the 60th day. Liver RNA level exhibited a significant decrease on the 30th ($P<0.05$), 45th ($P<0.001$) and 60th ($P<0.01$) days. However, decrease in the levels of ovarian RNA was noticed only on the 60th day of the experiment termination ($P<0.001$). RNA depletion in the brain ($r=-0.131$), liver ($r=-0.818$, $P<0.025$) and ovary ($r=-0.715$) was inversely correlated with exposure time (table 2).

DNA: Its content in the brain decreased significantly on the 60th day ($P<0.001$) of exposure whereas in liver it was lowered on the 45th ($P<0.01$) and 60th ($P<0.001$) days. Ovarian DNA content was decreased on 30th ($P<0.01$), 45th ($P<0.001$) and 60th ($P<0.05$) days. The DNA reduction was inversely correlated with exposure time and the correlation coefficient (r) values for brain, liver and ovary were -0.447, -0.823 ($P<0.025$) and -0.701, respectively (table 3).

Table 1 *Changes in the brain, liver and ovarian protein content (mg/g wet tissue) of fish exposed to 4.0 ppm of cythion for various exposure intervals. Values are the mean ± SD of five fish in treated and control groups*

Tissue/Treatment		Exposure time intervals (Days)				Correlation coefficient (r) value and regression line equation*
		15	30	45	60	
Brain	Control	82.50±7.65	80.40±7.89	84.22±7.84	86.61±2.42	$r=+0.859(P<0.01)$
	Treated	64.41±3.11 ($P<0.01$)	67.19±3.86 ($P<0.05$)	89.21±3.68 (NS)	84.47±3.62 (NS)	$Y=55.77+0.54X$
Liver	Control	133.71±5.35	136.13±3.97	137.53±4.13	139.88±10.20	$r=-0.666$ (NS)
	Treated	132.64±3.42 (NS)	133.50±8.46 (NS)	134.96±4.11 (NS)	121.72±4.79 ($P<0.05$)	$Y=138.52-0.20X$
Ovary	Control	108.34±4.01	106.86±9.02	108.56±8.34	106.58±9.94	$r=+0.095$ (NS)
	Treated	94.54±8.17 ($P<0.05$)	107.40±3.81 (NS)	103.95±6.45 (NS)	97.16±3.20 (NS)	$Y=99.66+0.02X$

*Regression line equation between changes in protein content (Y) and exposure time (X).

Table 2 *Changes in the brain, liver and ovarian RNA content (mg/g wet tissue) of fish exposed to 4.0 ppm of cythion for various exposure intervals. Values are the mean ± SD of five fish in treated and control groups*

Tissue/Treatment		Exposure time intervals (days)				Correlation coefficient (r) value and regression line equation*
		15	30	45	60	
Brain	Control	3.58±0.66	3.40±0.13	3.61±0.40	3.53±0.48	$r=-0.131$(NS)
	Treated	2.58±0.28 ($P<0.05$)	3.28±0.30 (NS)	3.25±0.15 (NS)	2.45±0.14 ($P<0.01$)	$Y=3.00-0.002X$
Liver	Control	12.25±0.44	12.11±0.29	12.00±0.47	11.99±0.51	$r=-0.818(P<0.025)$
	Treated	11.78±0.73 (NS)	11.10±0.79 ($P<0.05$)	9.82±0.64 ($P<0.001$)	10.41±0.73 ($P<0.01$)	$Y=12.12-0.03X$
Ovary	Control	30.82±0.45	30.28±0.63	29.53±1.82	30.45±2.03	$r=-0.715$(NS)
	Treated	28.08±2.48 (NS)	30.75±1.02 (NS)	27.81±1.14 (NS)	23.63±1.17 ($P<0.001$)	$Y=31.64-0.10X$

*Regression line equation between changes in RNA content (Y) and exposure time (X)

Table 3 *Changes in the brain, liver and ovarian DNA content (mg/g wet tissue) of fish exposed to 4.0 ppm of cythion for various exposure intervals. Values are the mean ± SD of five fish in treated and control groups*

Tissue/Treatment		Exposure time intervals (days)				Correlation coefficient (r) value and regression line equation*
		15	30	45	60	
Brain	Control	3.47±0.45	3.56±0.35	3.43±0.14	3.45±0.37	$r=-0.447$ (NS)
	Treated	2.92±0.49 (NS)	3.63±0.30 (NS)	3.51±0.29 (NS)	2.20±0.21 ($P<0.001$)	$Y=3.63-0.01X$
Liver	Control	5.21±0.29	5.23±0.44	5.20±0.27	5.09±0.18	$r=-0.823$ ($P<0.025$)
	Treated	5.02±0.22 (NS)	5.22±0.47 (NS)	3.92±0.66 ($P<0.01$)	4.04±0.31 ($P<0.001$)	$Y=5.60-0.02X$
Ovary	Control	3.24±0.28	3.36±0.43	3.46±0.40	3.36±0.24	$r=-0.701$ (NS)
	Treated	3.14±0.22 (NS)	2.56±0.09 ($P<0.01$)	2.35±0.18 ($P<0.001$)	2.60±0.44 ($P<0.05$)	$Y=3.11-0.01X$

*Regression line equation between changes in DNA content (Y) and exposure time (X)

Discussion

In general, results of the present investigation revealed that cythion induced reduction in the protein, RNA and DNA contents in different tissues of *C. punctatus*. OPPs are known to methylate and phosphorylate cellular protein (Wild 1975). Choudhari and Chakrabarti (1983) reported a decrease in serum and liver proteins in rats exposed to OPPs. Reduction of cytochrome oxidase activity is observed in different tissues of *Channa striatus* treated with OPP metasystox (Natarajan 1984). Dubale and Awasthi (1984) recorded time dependent depletion of protein content in liver and kidney of *Heteropneustes fossilis* treated with OPP dimethoate. The above authors observed decreased values during the first week which became normal by the end of third week. Dose dependent increase in serum protein levels in rat administered with tri-n-butyl phosphate is reported by Oishi et al. (1980). The protein content in the brain of cythion exposed *C. punctatus* decreased up to the 30th day and returned to normal level on the 45th and 60th days. In the ovary and liver significant reduction of protein was seen only on the 15th and 60th days, respectively. Mukhopadhyay and Dehadrai (1980 a,b) have reported decreased serum protein level and protein biosynthesis in liver in *Clarias batrachus* exposed to sublethal dose of malathion.

OPPs inhibit acid and alkaline phosphatase activity in different tissues of fishes (Dalela et al. 1978, Sastry & Malik 1979, Mukhopadhyay & Dehadrai 1980a, Sastry & Sharma 1980), which may adversely affect nucleic acid synthesis (Sastry & Sharma 1980). In *C. punctatus* exposed to cythion, RNA and DNA content of brain, liver and ovary were depleted significantly showing inverse correlation with exposure time. Patankar and Vaidya (1980) reported considerable genetic damages induced by phosphamidon in cultured human leucocytes and *in vivo* bone marrow cells of mice.

OPPs are known to inhibit cholinesterase activity in mallard ducklings (Fleming & Bradburg 1981). Gupta and Paul (1977) reported more accumulation of ^{32}P-labelled

malathion in liver than other organs of *Gallus domesticus*. In cythion treated *C. punctatus* significant positive correlation in brain protein reduction ($r = +0.859$, $P<0.01$), and inverse correlation in liver RNA ($r = -0.818$, $P<0.025$) and DNA ($r = -0.823$, $P<0.025$) contents depletion were noticed at different exposure intervals. These observations are suggestive of the primary action of cythion on brain during initial period of exposure and cumulative toxic effect in liver during prolonged exposure resulting subsequent more pronounced decrease of liver RNA and DNA. Thus cythion is capable of inducing alterations in the normal physiological functions in different organs possibly through impairing the synthesis and metabolism of protein, RNA and DNA in this species. Further, the toxicity seems to differ in relation to organs as well as biomolecules and the duration of exposure.

Acknowledgements

First author is grateful to the Council of Scientific and Industrial Research of India for the award of a Senior Research Fellowship, which made this work possible.

References

Anees M A 1978 Hepatic pathology in freshwater teleost *Channa punctatus* (Bloch) exposed to sublethal and chronic levels of three organophosphorus insecticides; *Bull. Environ. Contam. Toxicol.* **19** 524–527

Bruning J L and Kintz B L 1977 *Computational Handbook of Statisticals* (England: Scott, Foresman and Company — Glenview, Illinois) 171 pp

Choudhari P D and Chakrabarti C H 1983 Effect of isofenphos (Oftanol) and acephate (Orthene) on fecal bile acids, cholesterol and total protein content of serum and liver in albino rats; *Indian J. expl. Biol.* **21** 684–685

Dalela R C, Bhatnagar M C and Verma S R 1978 Histochemical studies on the effect of rogor and thiodon on the activity of acid phosphatase in liver, muscles and kidney of *Channa gachua; Indian J. expl. Biol.* **16** 1099–1102

Dubale M S and Awasthi M 1984 Biochemical changes in the liver and kidney of a catfish *Heteropneustes fossilis* exposed to dimethoate; *Comp. Physiol. Ecol.* **7** 111–114

—— and Shah P 1979 Histopathological lesions induced by malathion in the liver of *Channa punctatus; Indian J. exp. Biol.* **17** 693–796

Fleming W J and Bradburg S P 1981 Recovery of cholinesterase activity in mallard ducklings administered organophosphorus pesticides; *J. Toxicol. Environ. Health* **8** 885–897

Gupta P K and Paul B S 1977 Biochemical fate of ^{32}P-malathion in *Gallus domesticus* (Desai Poultry Birds); *Toxicology* **7** 169–177

Holden A V 1973 Effects of pesticides on fish; in *Environmental Pollution by Pesticides* 213–253 ed. C A Edwards (London & New York: Plenum Press)

Johnson D W 1973 Pesticides residue in fish; in *Environmental Pollution by Pesticides* pp 182–212 ed. C A Edwards (London & New York: Plenum Press)

Joshi U M and Desai A K 1981 Effects of sublethal concentration of monocrotophos on acid and alkaline phosphatase activities in the tissue of fresh water fish *Tilapia mossambica; J. Anim. Morphol. Physiol.* **28** 221–228

Lakota S, Raszka A, Roszkowski J, Hlond S, Kozlowski F and Stefan J 1982 Investigation on the toxicity of propoxur, chlorfenvinphos, bromfenvinphos and methylbromfenvinphos to the fry of the carp, *Cyprinus carpio*, using an acute test; *Folia Biol. (Krakow)* **30** 149–156

Lowry O H, Rosenbrough N J, Farı A L and Randall R J 1951 Protein measurement with the folin phenol reagent; *J. Biol. Chem.* **193** 265–275

Mandal P K and Kulshresthra A K 1980 Histopathological changes induced by the sublethal sumithion in *Clarias batrachus* (Linn.); *Indian J. exp. Biol.* **18** 547–552

Mukhopadhyay P K and Dehadrai P V 1980a Studies on air-breathing catfish *Clarias batrachus* (Linn.) under sublethal malathion exposure; *Indian J. exp. Biol.* **18** 400–404

—— and —— 1980b Biochemical changes in the air-breathing catfish *Clarias batrachus* (Linn.) exposed to malathion; *Environ. Pollut.* **A22** 149–158

Munro H N and Fleck A 1966 The determination of nucleic acids; in *Methods of Biochemical Analysis* Vol. 14 p 113 ed D Glick (New York: Interscience Publishers)

Natarajan G M 1983 Metasystox toxicity : Effects of lethal (LC_{50}/48 hr) concentration on free amino acids and glutamate dehydrogenase in some tissues in the air-breathing fish *Channa striatus* (Bleeker); *Comp. Physiol. Ecol.* **8** 254–256

—— 1984 Effects of lethal (LC_{50}/48 hr) concentration of metasystox on some selected enzyme systems in the air-breathing fish *Channa striatus* (Bleeker); *Comp. Physiol. Ecol.* **9** 29–32

Oishi H, Oishi S and Hiraga K 1980 Toxicity of Tri-n-butyl phosphate with special reference to organ weights, serum components and cholinesterase activity in male rats; *Toxicol. Lettrs.* **6** 81–85

Patankar N and Vaidya V G 1980 Evaluation of genetic toxicity of the insectide phosphamidon using *in vitro* and *in vivo* mammalian test systems; *Indian J. exp. Biol.* **18** 1145–1147

Sastry K V and Malik P V 1979 Studies on the effect of dimercron on the digestive system of a fresh water fish *Channa punctatus; Arch. Environ. Contam. Toxicol.* **8** 397–407

—— and Sharma K 1980 Diazinon effect on the activities of brain enzymes from *Ophiocephalus* (Channa) *punctatus; Bull. Environ. Contam. Toxicol.* **24** 326–332

Wild D 1975 Mutagenicity studies on organophosphorus insecticides; *Mutation Res.* **32** 133–150

Proc. Indian natn. Sci. Acad. B51 No. 5 pp 543–549 (1985)

Diffusing Capacity of Gills of a Freshwater Goby, *Glossogobius giuris* (Ham.)

O N SINGH and J S DATTA MUNSHI
Post-graduate Department of Zoology, Bhagalpur University, Bhagalpur 812007

(Received 16 July 1985; after revision 13 Novermber 1985)

The diffusing capacity of the gills of a fresh water goby, *Glossogobius giuris* (Ham.) has been determined in relation to body weight. The harmonic mean of the thickness of water-blood pathway was found to be 2.706 μm. The diffusing capacity Dt (ml O_2/min/mmHg) increases with increasing body weight by a power of 0.50873. As the slope of the diffusing capacity is less than 1, the weight-specific diffusing capacity Dt_1 (ml O_2/min/kg/mmHg) decreases with increasing body weight by a power of 0.50517.

Introduction

Diffusing capacity is an important physiological parameter as it gives an idea of the efficiency of the respiratory organ for oxygen transfer. The thickness of the water-blood pathways and effective gill area in fish are the important parameters to be considered in determining the perfusion capacity of gases through the respiratory interface. The water-blood pathways in the gills of fishes consist of outer epithelial cells, the middle basement membrane and the inner flanges of the adjacent pillar cells (Newstead 1967, Hughes & Grimstone 1965).

The thickness of water-blood diffusion barrier in the secondary lamellae of teleosts has been studied by many workers (Schulz 1960, Newstead 1967, Hughes & Munshi 1968, Hughes & Wright 1970, Hughes 1970, 1972, Dube & Munshi 1974, Ojha & Munshi 1976).

Weibel (1970, 1972) gave a detailed account of morphological estimation of pulmonary diffusing capacity of certain mammals. This method was then applied more elegantly by Hughes (1970, 1972). The calculated values for the diffusing capacity based on morphological measurements exceed those based on physiological measurements (Steen & Berg 1960, Hughes 1970, 1972).

The present investigation is an attempt to determine the possible relationship between the calculated diffusing capacity of gills and body weight in a freshwater goby, *Glossogobius giuris*.

Materials and Methods

The diffusing capacity of gills is determined from gill area (A) and thickness of water-blood barrier (t) with the help of modified

Fick's equation as follows (Hughes 1972, Weibel 1972).

$$\dot{V}o_2 = \frac{K.A.\triangle PO_2}{t} \quad \ldots (1)$$

$$or \quad \frac{\dot{V}o_2}{\triangle PO_2} = \frac{K.A.}{t} = \text{diffusing capacity (D}t\text{)} \quad \ldots(ii)$$

where, $\dot{V}o_2$ is the rate of oxygen consumption; K, Krogh's permeation coefficient for connective tissue of frog (Krogh 1919) and it is equal to 0.00015 ml $O_2/cm^2/\mu m/min/mmHg$; A, dimension of gill area; $\triangle PO_2$, difference of oxygen tension between water and blood; and t, thickness of water-blood pathway

Live specimens of *Glossogobius giuris* in the weight range of 3.2–19.0g (n= 8) were collected from local ponds and small pieces of 1st, 2nd, 3rd and 4th gill arches were fixed in Zenker's fixative and processed by the usual procedure. Paraffin sections cut at 6μm in the horizontal plane were stained with haemotoxylin+eosin combination. Photomicrographs were taken from various levels of the gill filaments of each gill arch and the maximum and minimum distances of water-blood pathway were measured from these photomicrographs and an average value was obtained. Arithmetic and harmonic means of the thickness of water blood pathway were determined. The data on the gill area of *G. giuris* have been taken from previous studies (unpublished data).

The values of gill dimensions and harmonic mean of the water-blood pathways were applied to the equation (ii) for the determination of diffusing capacity.

Regression analysis using logarithmic transformations was made to find out the relationship between the diffusing capacity of gills and body weight.

Transfer factor (TO_2) or diffusing capacity from physiologically measured oxygen uptake was also determined with the help of equation:

$$TO_2 = \dot{V}o_2/\triangle PO_2$$

Here the value of oxygen uptake ($\dot{V}o_2$) was taken from the previous study (unpublished data) assuming the pressure gradient ($\triangle PO_2$) as 100 mmHg.

Results

Water-blood Diffusion Distance

The thickness of the water-blood pathway varied from 1.371 to 6.723 μm. Arithmetic mean of the data obtained for the thickness was found to be 3.231 μm while harmonic mean ($\bar{X}h$) gave a lesser value, i.e., 2.706 μm. Harmonic mean of the thickness of the water-blood pathway was used for the calculation of diffusing capacity as described for tench (Hughes 1972).

Relationship between Body Weight and the Diffusing Capacity, Dt (ml O_2/min/mmHg):

The diffusing capacity of gills increased with increasing body weight in different and total gill arches. Log/log plots of the body weight and the diffusing capacity for 1st, 2nd, 3rd, 4th and total gill arches gave straight lines with slopes of 0.48778, 0.48107, 0.53587, 0.53429 and 0.50873 respectively. There was significant and positive correlation between the two variables.

Total diffusing capacity of a gill may be expressed by the following equations:

$$Dt = 0.000698W^{0.50873}$$

or $log\ Dt = log\ 0.000698 + 0.50873\ log\ W$

Relationship between Body Weight and Weight-specific Diffusing Capacity Dt_1 (ml O_2/min/mmHg/Kg)

The weight-specific diffusing capacity (Dt_1) ($ml O_2$/min/mmHg/kg) decreases with increasing body weight. In the log/log plots of the diffusing capacity (Dt_1) for

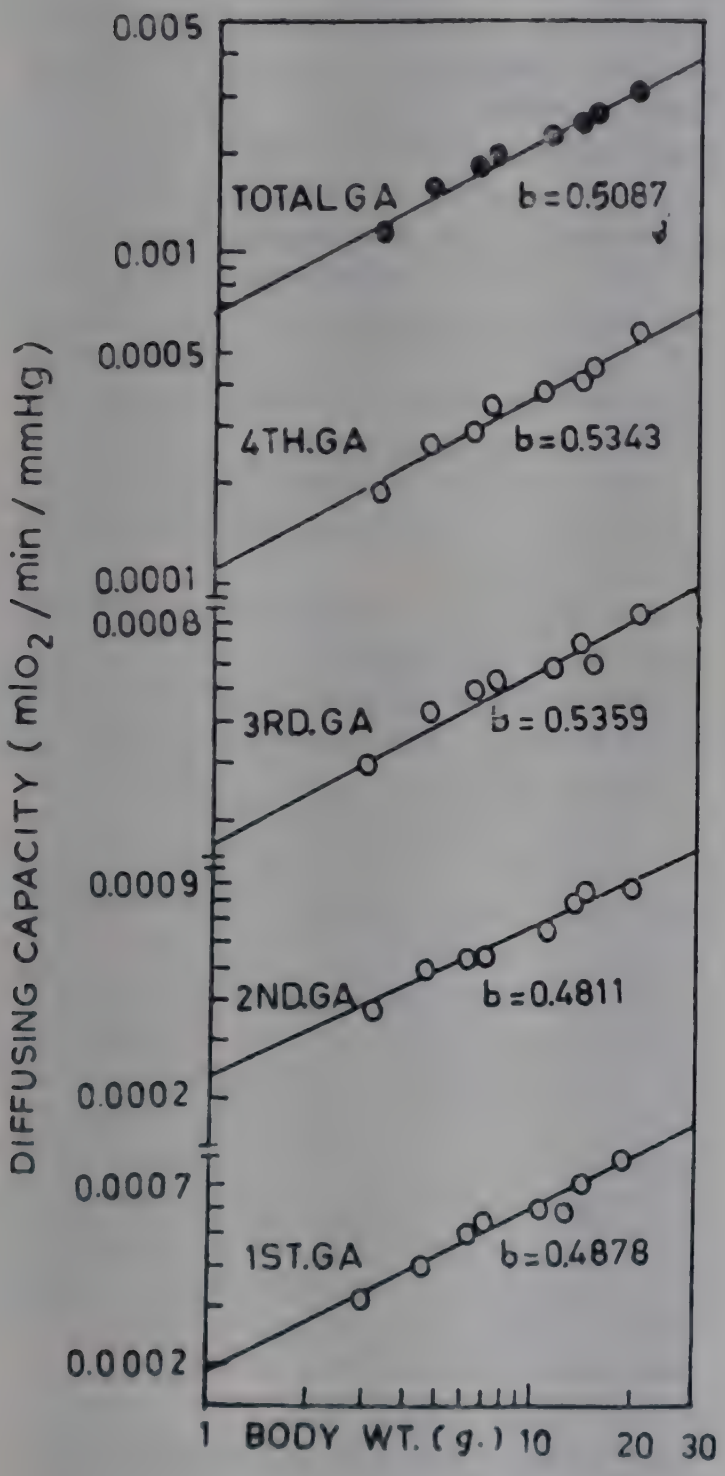


Figure 1 Log/log plots showing the relationship between body weight and diffusing capacity (Dt, ml O_2/min/mmHg) for different and total gill arches in *G. giuris*

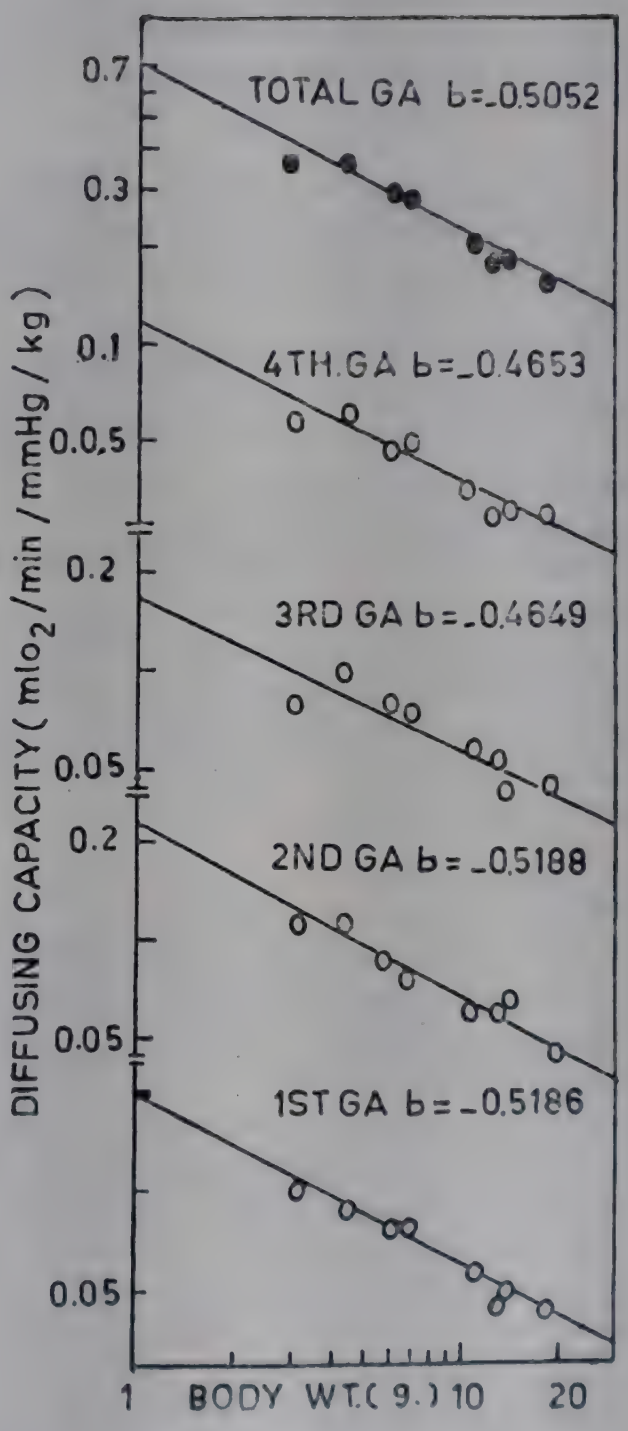


Figure 2 Log/log plots showing the relationship between body weight and weight specific diffusing capacity (Dt_1, mlO_2/min/mmMg/Kg) for different and total gill arches in *G. giuris*

different body weight groups, the slopes of regression lines were found to be —0.51855, —0.51872, —0.46493, —0.46534 and —0.50517 for 1st, 2nd, 3rd, 4th and total gill arches respectively.

The relationship between total diffusing capacity (Dt_1) and body weight can be expressed by following equation:

$$Dt_1 = 0.72205 W^{-0.50517}$$

The weight-specific diffusing capacity is also highly correlated with the body weight as indicated by the correlation coefficient value (r).

A summary of the estimated data for the diffusing capacity Dt_1 (ml O_2/min/mmHg/kg) of *G. giuris* for 1, 10 and 100g of fishes based upon the regression analysis using the least square method has been given in table 2 along with their 95% confidence limit.

Transfer factor (TO_2): The transfer factor of the gills for a particular gas O_2, a measure of the ability of the gills to transfer the oxygen per unit gradient, was estimated to be 0.00098 mlO_2/min/mmHg/kg.

Discussion

Hughes and Grimstone (1965), Newstead (1967) and Hughes and Wright (1970) found the diffusion barrier to be 3–6 μm in purely water-breathing teleosts. Munshi and Singh (1968) measured the water-blood pathway of gill of certain water-breathing and air-breathing teleosts of India. The value has been found as low as 0.533 to 0.598 for active fishes like Tunny (Hughes 1970). Later, Dube and Munshi (1974), Ojha and Munshi (1976), Hakim et al. (1978), Sharma et al. (1982) and Ojha et al. (1982) measured the water-blood pathway in different species of teleosts (table 3). In *G. giuris* the thickness of water-blood pathway ranged from 1.371 to 6.723μm with an average harmonic mean, 2.706μm. This value is higher than those reported for *Macrognathus aculeatum* (1.54μm), *Botia lohchata* (1.71μm), *Garra lamta* (1.75μm), *Mystus vittatus* (1.380μm) and *Cirrhinus mrigala* (1.290μm)—all Indian water-breather teleosts.

It is evident from the statistically computed data on the regression lines of the diffusing capacity (Dt) that in this goby diffusing capacity increases by a power of 0.50873 with unit increase in body weight. Variations in the slopes of the regression lines of different gill arches are due to corresponding changes in their lamellar surface area and indicate the heterogenous growth patterns of different gill arches. The weight-specific diffusing capacity (mlO_2/min/mmHg/kg) decreases with increasing body weight by a power of —0.50517. This indicates that the gills of younger fishes are more efficient than those of older ones.

The value of weight-specific diffusing capacity (Dt_1) for 100g of *G. giuris* was found to be 0.07050 mlO_2/min/mmHg/kg. This value is lower when compared to fishes of the same weight of other species (table 4), but higher than *Tinca tinca*, *Salmo gairdneri*, *A. testudineus*, *H. fossilis* and *Clarias batrachus*. These findings suggest a lesser oxygen uptake efficiency of this fish.

The diffusing capacity based on morphological measurements exceed those based on physiological measurements. It is because, in the aforesaid calculation the anatomical area has been considered which is a measure of maximum possible functional area, but the functional area of gills is less as it is based upon the blood channels only. Hughes (1966) estimated that the respiratory surface area was 60–70% of total lamellar surface area. Again there are alternate non-respiratory pathway and vascular shunts which can be used to decrease the lamellar blood flow (Steen & Kruysse 1964).

The morphometrically calculated $\dot{V}o_2$ for a 100g *G. giuris* was found to be 423.036 ml O_2/kg/hr. This value is much higher than the actual oxygen uptake (57.9 ml/kg/hr)

by this goby (Singh 1982). The morphometrically estimated oxygen uptake indicated the maximum oxygen uptake capacity of the fish. Besides, the fishes extract about 5–10 times more oxygen in active condition (Alexander 1967).

For a resting *G. giuris* of 100g the value of transfer factor (TO_2) (0.0098 ml O_2/min/mmHg/kg) is higher than that of a resting trout (0.0056 mlO_2/min/mmHg/kg; Randall et al. 1967) and dogfish (0.0080 ml O_2/min/mmHg/kg; Piper and Boumagarten-Schumann 1968), but lower than *C. mrigala* (0.01755 ml O_2/min/mmHg/kg; Roy 1983). Randall (1970) suggested that this factor is changed with change in functional area of the gills, diffusion distance and by change in the pattern of blood flow.

Table 1 *Showing Intercept (log a), slope (b) and correlation coefficient (r) for different and total gill arches. Their standard deviations (SD) are also given*

	Intercept (log a)		Slope (b)		Regression coeff. (r)
	Value	SD	Value	SD	
Dt (ml O_2/min/mmHg)					
1st gill arch	—3.71354	±0.01299	0.48778	±0.05197	0.92866 ($P<0.001$)
2nd gill arch	—3.64589	±0.01437	0.48107	±0.05781	0.95871 ($P<0.001$)
3rd gill arch	—3.77221	±0.02396	0.53587	±0.09639	0.91501 ($P<0.001$)
4th gill arch	—3.85387	±0.01429	0.53429	±0.05744	0.97709 ($P<0.001$)
Total gill arch	—3.15614	±0.01073	0.50873	±0.04318	0.97872 ($P<0.001$)
Dt_1 (ml O_2/min/mmHg/kg)					
1st gill arch	—0.70732	±0.00987	—0.51855	±0.03974	0.98704 ($P<0.001$)
2nd gill arch	—0.64572	±0.01453	—0.51876	±0.05848	—0.96403 ($P<0.001$)
3rd gill arch	—0.77024	±0.02389	—0.46493	±0.09618	—0.89217 ($P<0.001$)
4th gill arch	—0.95105	±0.01432	—0.46534	±0.04760	—0.95663 ($P<0.001$)
Total gill arch	—0.14143	±0.01170	—0.50517	±0.04707	—0.91347 ($P<0.001$)

Table 2 *Computed diffusing capacity for 1, 10 and 100 g fish along with 95% confidence limits*

Parameters	1g		10g		100g	
	Value	95% CL	Value	95% CL	Value	95% CL
Dt (ml O_2/min/mmHg)	0.00069	0.00056 0.00086	0.00223	0.00229 0.00219	0.00718	0.00950 0.00558
Dt_1 (ml O_2/min/mmHg/kg)	0.72205	0.58412 0.89255	0.22563	0.22976 0.22157	0.07051	0.09037 0.05501

Table 3 *Water-blood distance of gills for a number of freshwater and marine fishes*

Fish species	Water-blood distance			References
	Range	Arithmatic mean ($\bar{X}$)	Harmonic mean $\bar{X}h$)	
Katsuwonus pelamis		0.598	—	Hughes 1970
Scomber scombrus	0.600–3.63	1.215	—	–do–
Thunnus albacares	0.166–1.13	0.533	—	–do–
Trachurus trachurus	0.250–3.13	0.533	—	–do–
Onchorhynchus kisutch	0.640–3.50	—	—	Newstead 1967
Carassius carassius	0.500–0.60	—	—	Schulz 1960
Barbus stigma	0.800–3.20	—	—	Munshi & Singh 1968
Catla catla	0.800–3.20	—	—	–do–
Salmo gairdneri	3.20 –9.60	6.37	–	Hughes 1970
Tinca tinca	1.600–3.50	2.47	—	–do
Macrognathus aculeatum	0.500–3.00	1.54–2.25	1.39–1.98	Ojha & Munshi 1976
Mystus cavasius	1.200–3.20	—	2.15	Singh 1979
Mystus vittatus	0.900–2.00	—	1.38	–do–
Botia lohachata	—	—	1.71	Sharma et al. 1982
Garra lamta	—	—	1.75	Ojha et al. 1982
Cirrhinus mrigala	0.675–3.42	1.752	1.290	Roy 1983
Glossogobius giuris	1.371–6.723	3.231	2.706	Present authors

Table 4 *Diffusing capacity (ml O_2/min/mmHg/kg) of gills of different fish species*

Fish Species	Body wt (g)	Diff. Cap.	References
Tinca tinca	100	0.0525	Hughes 1970
Salmo gairdneri	100	0.0500	Hughes 1972
Tuna	100	6.0000	Hughes & Gray 1972
Opsanus tau	100	0.0630	–do–
Anabas testudineus	100	0.0071	Hughes et al. 1973
Heteropneustes fossilis	100	0.0242	Hughes et al. 1974
Channa punctata	100	0.0530	Hakim et al. 1978
Mystus vittatus	100	0.2111	Singh 1979
M. cavasius	100	0.2910	–do–
Clarias batrachus	100	0.0162	Munshi et al. 1980
Botia lohachata	1	0.8000	Sharma et al. 1982
Garra lamta	4.1	0.02759	Ojha et al. 1982
Cirrhinus mrigala	100	0.5891	Roy 1983
Glossogobius giuris	100	0.0705	Present authors

References

Alexander R M 1967 Respiration; in *Functional Design in Fishes* pp 65–88 (London: Hutchinson and Co Ltd)

Dube S C and Munshi J S D 1974 Studies on the blood water diffusion barrier of secondary gill lamellae of an airbreathing fish, *Anabas testudineus*; *Zool. Anz.* **193** 35–41

Hakim A, Munshi J S D and Hughes G M 1978 Morphometrics of the respiratory organs of the Indian green snake headed fish, *Channa punctata*; *J. Zool. Lond.* **184** 519–543

Hughes G M 1966 The dimensions of fish gills in relation to their function; *J. exp. Biol.* **45** 177–195

—— 1970 Gill dimensions in relation to other respiratory parameters; *Proc. 19th Int. Congr. Anat.* Leningrad

—— 1972 Morphometrics of fish gills; *Respir. Physiol.* **14** 1–26

—— and Gray I E 1972 Dimensions and ultrastructure of toad fish gills; *Biol. Bull. mar. biol. lab., Woods Hole* **143** 150–161

——, Dube S C and Munshi J S D 1973 Surface area of the respiratory organs of the climbing perch, *Anabas testudineus*; *J. Zool. Lond.* **170** 227–243

—— and Grimstone A V 1965 The fine structure of the secondary lamellae of the gills of *Gadus pollachius*; *Q. J. Mic. Sci.* **106** 343–353

—— and Munshi J S D 1968 Fine structure of the respiratory surfaces of an air-breathing fish, the climbing perch, *Anabas testudineus* (Bloch); *Nature* **219** 1382–1384

——, Singh B R, Guha G, Dube S C and Munshi J S D 1974 Respiratory surface areas of an air-breathing siluroid fish, *Saccobranchus* (=*Heteropneustes*) *fossilis*; *J. exp. Biol.* **55** 667–682

—— and Wright D E 1970 A comparative study of the ultrastructure of the water-blood pathway in the secondary lamellae of teleost and elasmobranch fishes, benthic forms; *Z. Zellforsch* **104** 478–493

Munshi J S D and Singh B N 1968 A study of the gill epithelium of certain freshwater teleostean fishes with special reference to the air-breathing fishes; *Ind. J. Zootomy* **9** (3) 91–107

——, Ojha J and Sinha A L 1980 Morphometrics of the respiratory organs of an air-breathing catfish, *Clarias batrachus* (Linn.) in relation to body weight; *Proc. Indian natn. Sci. Acad.* **B46** 621–635

Newstead J D 1967 Fine structure of the respiratory lamellae of teleostean gills; *Zeitschrift fur Zellforschung* **79** 396–428

Ojha J and Munshi J S D 1976 Morphometric estimation of gill diffusing capacity of a freshwater mud eel, *Macrognathus aculeatum* (Bloch) in relation to body weight; *Zool. Beitr.* **22** 87–98

——, Rooj N C and Munshi J S D 1982 Dimensions of the gills of an Indian Hill-stream cyprinid fish; *Garra lamta*; *Jap. J. Ichthyol.* **29** 272–278

Piper J and Boumagarten-Schumann D 1968 The effectiveness of O_2 and CO_2 exchange in the gills of the dogfish (*Scyliorhinus stellaris*); *Respir. Physiol.* **5** 338–349

Randall D J 1970 Gas exchange in fish, in *Fish Physiology* Vol. IV pp 253–292 ed. Hoar and Randall (New York: Academic Press)

Roy P K 1983 Morphometrics of the Respiratory Organs of Certain Major Carp. PhD Thesis, Bhagalpur University, Bhagalpur

Schulz H 1960 Die submikroscopi sche Morphologie des Kiemenepithels; in *Proc 4th Conf. Electron Microscopy, Berlin* 1958 **2** 421–426

Sharma S N, Guha G and Singh B R 1982 Gill dimensions of a hill-stream fish, *Botia lohachata* (Pisces: Cobitidae); *Proc. Indian natn. Sci. Acad.* **B48** 81–91

Singh O N 1982 Biology of certain Gobiid fish of India, PhD Thesis, Bhagalpur University, Bhagalpur

Singh R 1979 Interspecific variation in the functional capacity of the respiratory organs of certain siluroid fishes of the genus *Mystus*; PhD Thesis, Bhagalpur University, Bhagalpur

Steen J B and Berg T 1960 The gills of two species of haemoglobin-free fishes compared to those of other teleosts, with a note on severe anaemia in eel; *Comp. Biochem. Physiol.* **18** 517–526

Steen J B and Kruysse A 1964 The respiratory function of teleostean gills; *Comp. Biochem. Physiol.* Vol. **12** 127–142

Weibel E R 1970 Morphometric estimation of pulmonary diffusion capacity. I. Model and Method; *Respir. Physiol.* **11** 54–75

—— 1972 Morphometric estimation of diffusion capacity. V. Comparative morphometry of alveola lungs; *Respir. Physiol.* **14** 26–43

Proc. Indian natn. Sci. Acad. B51 No. 5 pp 550–554 (1985)

Allometric Studies in *Macrobrachium scabriculum* (Heller, 1862)

K V JAYACHANDRAN and N I JOSEPH
Department of Aquatic Biology & Fisheries, University of Kerala, Beach P.O., Trivandrum 695007

(Received 2 April 1985)

Allometric studies of a few important taxonomic characters, carapace length, length of telson, length of rostrum and width of carapace of *Macrobrachium scabriculum* have been worked out in detail. Of these characters, lengths of carapace and telson are related to total length and length of rostrum and width of carapace with carapace length. The growth pattern of these characters significantly differ in the two sexes according to the analysis of covariance method and 't' test. Therefore, regression equations have been calculated separately for the two sexes for the above characters and a combined equation for width of carapace.

Key Words: **Allometry,** ***Macrobrachium scabriculum*****, Analysis of covariance, Regression coefficient, Body proportion index**

Introduction

Though information on the biology of *Macrobrachium*, a commercially important genus is vast (see Jayachandran 1984 for review), allometric studies in confirming the status of different species are limited (Tazelaar 1930, Tiwari 1962, 1963, Rao 1967, Koshy 1969, 1971). Therefore, an attempt has been made here to study the growth patterns of some body parts of taxonomic importance in one species of this genus, *M. scabriculum*.

Materials and Methods

A total of 196 specimens of *M. scabriculum* (116 males, ranging in total length from 24 to 63 mm and 80 females, ranging in total length from 25 to 45 mm) were collected from Vellayani Lake, Trivandrum. Total length (TL), carapace length (CL) and length of rostrum (LR) were recorded based on the method of Koshy (1971). Width of carapace (WC) was taken as the maximum horizontal width of the carapace and length of telson (LT) was measured from the base to the tip of telson. The carapace length and length of telson were related with total length and width of carapace and length of rostrum with carapace length.

Two methods have been employed in the present study: (*i*) analysis of covariance to compare the growth patterns of the various morphometric characters (Snedecor & Cochran 1975) and (*ii*) body proportion index using statistical 't' test (Pillai 1951).

Results

Analysis of Covariance Method

(a) *Characters related with total length*: Results show that the characters, carapace length and length of telson showed significant difference at elevations (Fe values : CL=13.3642, significant at 1% level; LT=6.3961, significant at 5% level). It may be inferred that the growth patterns of the two characters are different in the two sexes. Therefore, regression equations have been calculated separately for the two sexes for each character which are graphically presented in figures 1 & 2. Regression coefficients and average size of body measurements are presented in table 1. The growth rate and average size of the two characters are greater in males than females.

(*b*) *Characters related with carapace length*: On analysis length of rostrum alone showed significant difference at elevations whereas the width of carapace did not show any significant difference either in slope or elevation (Fe values : LR=29.2209, significant at 1% level; WC=0.0295, not significant). Therefore, regression equations have been calculated separately for the sexes in the case of length of rostrum and a combined equation for width of carapace (figures 3 & 4). Regression coefficients and average sizes of the two characters are given in table 1.

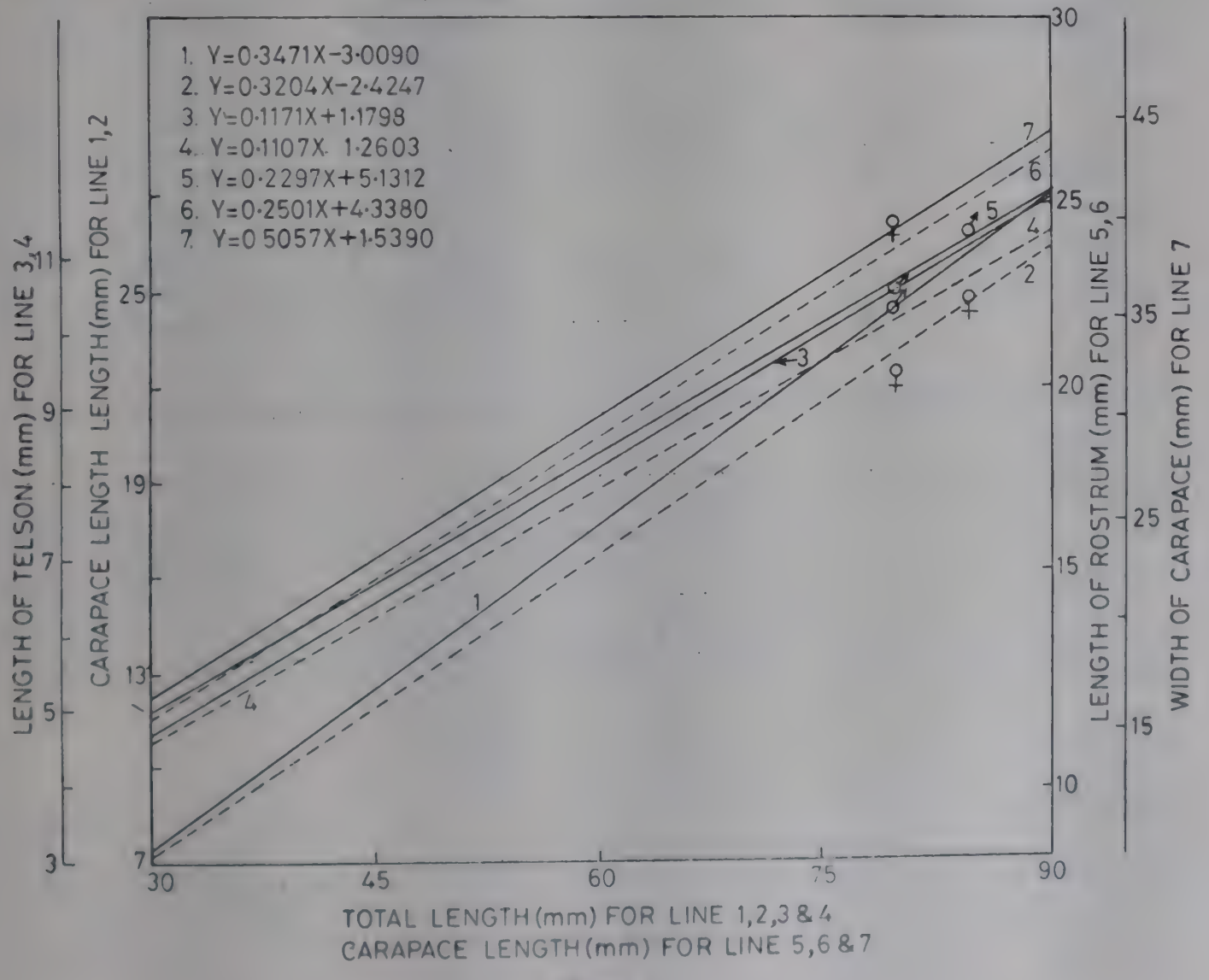


Figure 1

Body Proportion Index Method

(*a*) *Characters related with total length*: The proportions of carapace length alone showed significant difference between the sexes (greater in males than in females), whereas the proportions of the two sexes are almost same in both sexes (table 2).

(*b*) *Characters related with carapace length*: The proportion of both width of carapace and length of rostrum are significant and the females have a greater proportion than males (table 2).

Discussion

The overall shape, length, disposition of the teeth on the rostrum, telson and carapace have always been and continued to be some of the important taxonomic features. The study of growth pattern of these characters are, therefore, useful in confirming the status of a species and also establishing sexual dimorphism.

During the present study when analysis of covariance was applied, it was found that carapace length and length of telson in

Table 1 *Regression coefficients (Growth rates) and average size of body measurements for males and females of* M. scabriculum

Rate of growth and average size compared to total length and carapace length of M. scabriculum

Sl. No.	Morphometric characters	Regression coefficients		Mean values	
		Male	Female	Male	Female
1.	Carapace length	0.3471	0.3204	12.9397	9.9187
2.	Length of telson	0.1171	0.1107	6.5603	5.5250
3.	Width of carapace	0.5252	0.4267	8.0776	6.5625
4.	Length of rostrum	0.2297	0.2501	8.1034	6.8187

Table 2 *Comparison of body proportion indices in relation to total length and carapace length between sexes of* M. scabriculum

Morphometric characters	Mean values of morphometric characters as percentage to the total length		Mean values of morphometric characters as percentage to carapace length	
	Male	Female	Male	Female
Carapace length	28.02± 0.17	25.68±(*) 0.24	—	—
Length of telson	14.35± 0.10	14.37± 0.18	—	—
Width of carapace	—	—	62.86±(*) 0.51	66.66± 0.72
Length of rostrum	—	—	64.14±(*) 0.87	69.73± 1.10

(*) $P < 0.01$

relation to total length and length of rostrum in relation to carapace length showed significant difference in growth pattern between sexes. With 't' test, carapace length in relation to total length and length of rostrum and width of carapace in relation to carapace length showed significant difference in growth pattern between sexes.

Cole (1958) reported that the relation between carapace length and total length in *Palaemon serratus* was found to differ slightly according to age and sex of prawns. Here the mean values of the ratio of total length and carapace length were 5.46 and 5.22 in males and females respectively. Rajyalekshmi (1980) expressed the relationship between total length to carapace length and found that males and females of *M. malcolmsonii* of Godavari and Hooghly river systems showed difference in growth pattern between sexes. Brusher (1972) studied the relationship between total length and tail length of the prawn, *Penaeus indicus* and found that females showed slightly higher growth pattern. In *M. scabriculum* the growth pattern of carapace length and length of telson in relation to total length were significant. Here males show faster growth rate of carapace length than females as in *Palaemon serratus* but the growth rate of length of telson contrasts to *Penaeus indicus*.

Koshy (1969) worked out the rate of growth of the rostrum in relation to carapace length of *Macrobrachium lamarrei* and found that the growth rate was higher in females than males. According to him greater range of the measurements of females to males is due to the fact that females grow to much larger size than males. On the other hand, in many species of *Macrobrachium*, *M. rosenbergii*, *M. malcolmsonii*, *M. dayanus*, it is the male which grow to a larger size than female. In *M. lamarrei* it was found that the length of rostrum of females was 40% longer than males. In *M. scabriculum* the regression coefficient of length of rostrum is not significantly different between sexes but the values are slightly larger in females than males. Similarly, Koshy (1971) reported that in *M. dayanus* the regression coefficient differ significantly in carapace length, length of rostrum and length of the first cheliped in relation to the length of cephalothorax between the sexes, thereby establishing sexual dimorphism. The present results indicate that *M. scabriculum* not only exhibits clear sexual dimorphism but also possesses specific growth patterns characteristic to the species. This data is useful for taxonomic purposes.

Acknowledgements

The authors are thankful to Shri N K Balasubramanyam, Lecturer in biostatistics, for the statistical interpretations of the paper. They are also grateful to Padmasree N Balakrishnan Nair, Head of the Department of Aquatic Biology & Fisheries for the constant encouragement and facilities provided in carrying out the present work. One of us (KVJ) thanks UGC for awarding a research fellowship, during the tenure of which the present work was carried out.

References

Brusher H A 1972 Tail length-total length relation for the commercially important prawn; *Penaeus indicus*; *Indian J. Fish.* **19** 180–182

Cole H A 1958 Notes on the biology of the Common prawn *Palaemon serratus* (Pennant); *Fishery Invest.* **22** (ser. 2 No. 5) 1–22

Jayachandran K V 1984 Studies on the Biology of the Palaemonid Prawns of the South-west Coast of India; Ph.D. Thesis, Univ. of Kerala

Koshy M 1969 On the sexual dimorphism in the freshwater prawn *Macrobachium lamarrei* (H. Milne Edwards, 1837) (Decapoda, Caridea); *Crustaceana* **16** 185–193

—— 1971 Studies on the sexual dimorphism in the freshwater prawn *Macrobrachium dayanus* (Henderson, 1893) (Decapoda, Caridea). Part I; *Crustaceana* **21** 72–78

Pillai T V R 1951 A morphometric and biometric study of the systematics of certain allied species of the genus–*Barbus* Cuv. & Val.; *Proc natn. Inst. Sci. India* **17** (5)

Rajyalekshmi T 1980 Comparative study of the biology of the freshwater prawn, *Macrobrachium malcolmsonii* of Godavari and Hooghly river systems; *Proc. Indian natn. Sci. Acad.* **B46** 72–89

Snedecor G W and Cochran W G 1975 *Statistical Methods* (New Delhi: Oxford & IBH Publishing Co.)

Tazelaar M A 1930 The relative growth of parts of *Palaemon carcinus*; *Journ. exp. Biol.* **7** 165–174

Tiwari K K 1962 Occurrence of the freshwater prawn *Macrobrachium latimanus* (Von Martens) in India and Ceylon; *Crustaceana* **3** 98–104

—— 1963 A note on the freshwater prawn, *Macrobrachium altifrons* (Henderson, 1893) (Crustacea : Decapoda: Palaemonidae); *Proc. Zool. Soc. Calcutta* **16** 225–238

Proc. Indian natn. Sci. Acad. B51 No. 5 pp 555-559 (1985)

Modulation in Nitrogen Metabolism of Freshwater Mussel, *Lamellidens marginalis* (Lamarck) under Methyl Parathion Stress

B KASI REDDY, K S SWAMI and C S CHETTY
Department of Zoology, S V University, Tirupati 517502

(Received 3 June 1985; after revision 7 October 1985)

Effect of sub-lethal (8 ppm) methyl parathion (MP), an organophosphate (OP), on nitrogen metabolism of *L. marginalis* has been studied. An increase in the levels of NAD dependent glutamate dehydrogenase (GDH), glutamine synthetase, arginase, AMP deaminase and adenosine deaminase activities were observed in most of the tissues. The foot and hepatopancreatic GDH activity level, however was diminished. Ammonia, urea and glutamine were elevated in the tissues studied.

Key Words: Methyl parathion, Urea, Ammonia, *L. marginalis*

Introduction

Very few reports are available on the effects of organophosphate (OP) compounds on nitrogen metabolism in non-target organisms (Gupta & Paul 1978, Michail et al. 1979). Studies in this laboratory on changes in carbohydrate metabolism of the tissues of freshwater mussel during methyl parathion toxicity have suggested the possible alterations in energy metabolism and nitrogen metabolism (Sreenivasamoorthy et al. 1985). Hence in the present investigation an attempt has been made to explore certain aspects of ammonia production and utilization in freshwater mussel exposed to sub-lethal concentration of methyl parathon (MP).

Materials and Methods

Lamellidens marginalis (30±5 g) were collected from local freshwater ponds and fed *ad libitum* with freshwater plankton. Prior to use they were acclimatized to the laboratory conditions for 15 days and starved before the day of experimentation (Jones 1972). The 48 h LC_{50} concentration (25 ppm) was previously determined by probit analysis (Finney 1969) and 1/3 of this concentration (8 ppm) was selected for the present investigation. The mussels were exposed to sub-lethal concentration (8 ppm) of methyl parathion for 72 h. After stipulated time, the tissues were isolated and 5% homogenates were prepared in distilled water

for AMP deaminase activity, glutamine, urea and ammonia, malate buffer for adenosine deaminase, Tris-HCl buffer for glutamine synthetase, cetyltrimethyl ammonium bromide for agrinase and 0.25 M sucrose for GDH. The homogenates were centrifuged at 1000 × g for 15 min. and the supernatant was used for estimation of various enzymes.

NAD dependent - Glutamate dehydrogenase (GDH): The GDH assay was estimated by the method of Lee and Lardy (1965). The reaction mixture in a total volume of 2.0 ml contained 100 μ moles of sodium phosphate buffer (7.2 *p*H), 40 μ moles of sodium glutamate, 2 μ moles of INT and 75–100 μg of protein as an enzyme source. The reaction mixture was incubated for 30 min at 37°C. The reaction was arrested by 5 ml of glacial acetic acid and the formazan formed was read at 495 nm against reagent blank in spectrophotometer.

AMP-deaminase: The reaction mixture in a final volume of 2.0 ml contained 50 μmoles of malate buffer (7.0 *p*H), 10 μmoles of AMP, 5 μmoles of ATP, 2.5 μmoles of $MgCl_2$ and 10 μmoles of EDTA. The reaction was initiated by 120 μg of protein as an enzyme source and incubated at 37°C for 30 min. Ammonia formed was estimated by the method of Weil-Malherbe and Green (1955) with slight modification of Waegelin et al. (1978).

Adenosine deaminase: The reaction mixture in a total volume of 2.0 ml contained 50 μmoles of malate buffer (6.5 *p*H), 20 μmoles of adenosine and 250 μg protein as an enzyme source. The reaction mixture was incubated for 30 min at 37°C and the reaction was arrested by adding 2 ml of 0.1M phenol pentacyanonitrosyl ferrate. The ammonia thus formed was determined by Galanti and Glusti (1974).

Arginase: Arginase activity was estimated by the method of Campbell (1961), the reaction mixture in a final volume of 2.0 ml contained 50 μ moles of sodium glycinate buffer (9.5 *p*H), 0.5 μmoles of $MnCl_2$ and 200 μg of protein. The reaction was incubated at 37°C for 30 min. The reaction was stopped by 4 ml of 0.5 M PCA. The urea formed was estimated by the method of Natelson (1971).

Glutamine synthetase: The reaction mixture in a total volume of 4.5 ml contained 200 μmoles of L-glutamate, 200 μmoles of hydroxylamine hydrochloride, 4.0 μmoles of $MnCl_2$, 50 μmoles of L-cysteine, 20 μmoles of ATP and 200 μg protein as an enzyme source. The reaction was incubated for 30 min at 37°C. The reaction was arrested by adding 50% TCA. The gutamyl hydroxamate formed was estimated by the method of Wu (1963).

Glutamine, urea and ammonia were estimated by the methods of Colowick and Kaplan (1971), Natelson (1971) and Bergmeyer (1965). The protein content was determined by method of Lowry et al. (1951). All the enzymes were assayed after due standardization of various parameters such as enzyme concentration, incubation time, substrate concentration (V_{max} and K_m), *p*H and temperature. The data were analyzed by the student '*t*' test to assess difference between control and experimental treatment. A value of a $P<0.05$ was considered as a significant.

Results and Discussion

In general, the levels of AMP deaminase and adenosine deaminase activities were found to be increased significantly in the tissues of mussel exposed to MP for 72 hr (table 2). This indicates augmented purine catabolism resulting in an elevation of ammonia. The activity levels of GDH, a mitochondrial enzyme was increased in the mantle and gill and diminished in hepatopancreas and foot. The increased

Table 1 *Levels of ammonia, urea and glutamine in the tissues of control and methyl parathion exposed mussels. Values are expressed as μmoles/gr wt wet of tissue*

Metabolite	Hepatopancreas		Foot		Mantle		Gill	
	Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt.
Ammonia	7.91 ±0.34	11.33* ±0.47 (+43.24)	3.26 ±0.16	5.48* ±0.20 (+68.10)	6.27 ±0.20	10.40* ±0.46 (+65.87)	4.78 ±0.32	8.25* ±0.38 (+72.59)
Urea	1.01 ±0.26	1.48* ±0.19 (+46.53)	0.05 ±0.01	0.07* ±0.01 (+41.67)	0.70 ±0.07	0.840* ±0.07 (+20.26)	0.82 ±0.02	1.10 ±0.08 (+33.50)
Glutamine	0.013 ±0.006	0.021* ±0.003 (+62.77)	0.063 ±0.031	0.008* ±0.043 (+44.72)	0.46 ±0.07	0.70* ±0.09 (+52.50)	0.20 ±0.04	0.24* ±0.04 (+24.62)

Each value is mean ± SD of six individual observations.
*Significantly different from control ($P<0.05$). Values in parentheses are % changes over control

Table 2 *Activity levels of AMP and adenosine deaminase, GDH, glutamine synthetase and arginase in tissues of control and methyl parathion exposed mussels*

Enzyme	Hepatopancreas		Foot		Muscle		Gill	
	Control	Expt.	Control	Expt.	Control	Expt.	Control	Expt.
AMP-deaminase (μ moles of ammonia formed/ mg protein/h)	0.013 ±0.006	0.021* ±0.003 (+70.63)	0.063 ±0.031	0.108* ±0.043 (+72.06)	0.039 ±0.008	0.068 ±0.004 (+71.58)	0.025 ±0.002	0.041* ±0.006 (+62.80)
Adenosine deaminase (μ moles of ammonia formed/mg protein/h)	0.699 ±0.016	1.045* ±0.077 (+49.50)	0.413 ±0.030	0.464* ±0.006 (+12.35)	0.396 ±0.021	0.578* ±0.024 (+45.96)	0.085 ±0.022	0.148* ±0.013 (+60.80)
GDH (μ moles of formazan formed/mg protein/h)	0.994 ±0.093	0.484* ±0.014 (—51.26)	0.360 ±0.057	0.209* ±0.016 (—41.94)	0.364 ±0.053	0.424* ±0.023 (+16.05)	0.224 ±0.012	0.287* ±0.032 (+30.00)
Arginase (μ moles of urea formed/ mg protein/h)	0.640 ±0.044	1.010* ±0.053 (+57.81)	0.184 ±0.024	0.222* ±0.006 (+20.65)	0.091 ±0.007	0.149* ±0.008 (+63.74)	0.032 ±0.016	0.044* ±0.001 (+37.50)
Glutamine synthetase (μ moles of Y. glutamyl hydroxamate/mg protein/h)	0.540 ±0.037	0.930* ±0.038 (+72.20)	0.030 ±0.002	0.039* ±0.002 (+30.29)	0.032 ±0.016	0.044* ±0.001 (+36.79)	0.049 ±0.017	0.057* ±0.013 (+15.54)

Each value is mean ±SD of 6 individual observations. *Significantly different from control ($P<0.05$). Values in parentheses are % changes over control

GDH in gill and mantle indicates increased glutamate oxidation resulting in ammonia production (Ahmad 1979) and decreased GDH activity in hepatopancreas and foot suggests decreased oxidation of glutamate (Natarajan 1983). In evidence to this, the glutamate levels were found to be increased in the hepatopancreas and foot and decreased in mantle and gill under MP stress (Kasi Reddy 1984).

Ammonia, a byproduct of purine and amino acid catabolism is a toxic compound (Lowenstein 1972). The cells are intolerable to high concentration of ammonia. In the present investigation, ammonia levels were found to be elevated in the tissues studied (table 1). The elevation was more in hepatopancreas when compared to other tissues. The enzymes such as arginase and glutamine synthetase involved in the synthesis of two nitrogenous substances namely urea and glutamine were studied. Hepatopancreatic arginase was high and also increased significantly as compared to other tissues studied. Glutamine synthetase, a mitochondrial enzyme was also elevated under MP stress and the elevation was more in hepatopancreas as compared to other tissues. In consonance to this observation, glutamine and urea levels were found to be increased in all the tissues studied and the increase was significantly in hepatopancreas. The presence of urea and glutamine in non-hepatic tissues could be due to its transport from the hepatopancreas. This study indicates that MP induced the triggering of ammoniagenesis. To avert the additional toxicity of ammonia, the tissues have mobilized this toxic metabolite into the synthesis of safer products like urea and glutamine as evidenced through the increased specific activities of arginase and glutamine synthetase respectively.

The present investigation concludes that ammoniagenesis was triggered by increased deamination of purines and oxidative deamination of glutamate under MP stress. This results in the production of ammonia. As a consequence of this, the tissues adopted the mechanisms to detoxify the ammonia by enhancing the synthesis of urea and glutamine and the observed changes in nitrogen metabolism under methyl parathion were only secondary.

Acknowledgements

Dr B K R is grateful to the University Grants Commission, New Delhi, for providing financial support through Senior Research Fellowship.

References

Ahmad K I 1979 Studies on some aspects of protein metabolism and associated enzymes in the freshwater teleost, *Sarotherodon mossambicus* subjected to malathion exposure, PhD Thesis, S V University, Tirupati

Bergmeyer H U 1965 *Methods of Enzymatic Analysis* ed. H U Bergmeyer (New York: Academic Press)

Campbell J W 1961 Studies on tissue arginase and ureogenesis in the elasmobranch, *Mystelis canis*; *Arch. Biochem. Biophys.* **93** 448–455

Colowick S P and Kaplan N O 1971 *Methods in Enzymology* (USA: Academic Press) 728 pp

Finney D J 1969 *Probit Analysis* (London: Cambridge University Press)

Galanti S and Glusti G 1974 *Methods of Enzymatic Analysis* 2nd edn. ed. H U Bergmeyer (New York: Academic Press) 260 pp

Gupta R C and Paul B S 1978 Influence of malathion on body enzymes in dermal subacute toxicity studies in *Bubalus babalis* species; *Bull. Environ. Contam. Toxicol.* **20** 811–818

Jones J R E 1972 *River Pollution* 2. *Causes and Effects* ed. K L Butterworth (New York: Academic Press) 254 pp

Kasi Reddy B 1984 The possible metabolic diversions in nitrogen metabolism adopted by freshwater mussel, *Lamellidens marginalis* (Lamarck) under short term methyl parathion stress. PhD Thesis, S.V. University, Tirupati, India

Lee Y L and Lardy H A 1965 Influence of thyroid hormones on L. glycerophosphate dehydrogenase and other dehydrogenases of various organs of rats; *J. Biol. Chem.* **240** 1427–1431

Lowenstein J M 1972 Ammonia production in muscle and other tissues: The purine nucleotide cycle; *Physiol. Rev.* **52** 381–413

Milchail T H, Aggour N, Awandallah M W, Boulos E A, E L Dessovkey A, Karima A I 1979 Acute toxicity of organophosphorous and insecticides in laboratory animals; *Z. Ernachrung Swiss* **18** 258–268

Natarajan G M 1983 Metasystox toxicity. Effect of lethal LC_{50}/48 h concentration on free amino acids and glutamate dehydrogenase in some tissues in the air-breathing fish, *Channa striatus* (Blecker); *Comp. Physiol. Ecol.* **8** 254–256

Natelson S 1971 *Techniques of Clinical Chemistry* ed. C G Charles (Illinois, USA: Thomas Publication Co)

Wegelin I, Manzoli F A and Pane G 1978 Enzymes involved in AMP metabolism in chick embryo muscles; *Comp. Biochem. Physiol.* **59B** 55–57

Weil Malherbe H and Green R H 1955 Ammonia formation in brain. Studies on slices and suspensions; *Biochem. J.* **61** 210–218

Wu C 1963 Glutamine synthetase-1. A comparative study of its distribution in animals and its inhibition by dl Allo-o-hydroxyl lysine; *Comp. Biochem. Physiol.* **8** 335–351

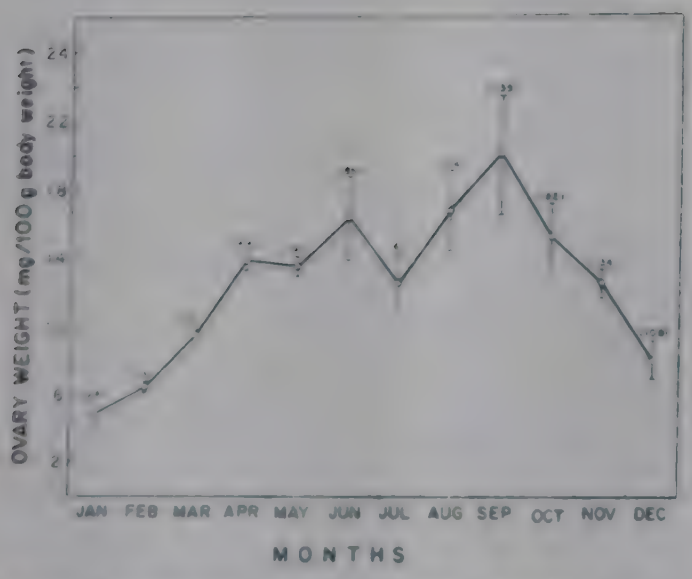


Figure 1 Monthly changes in ovarian weight in adults. Each point represents the mean value and the vertical lines show SEM. The figures in parenthesis indicate the number of animals used

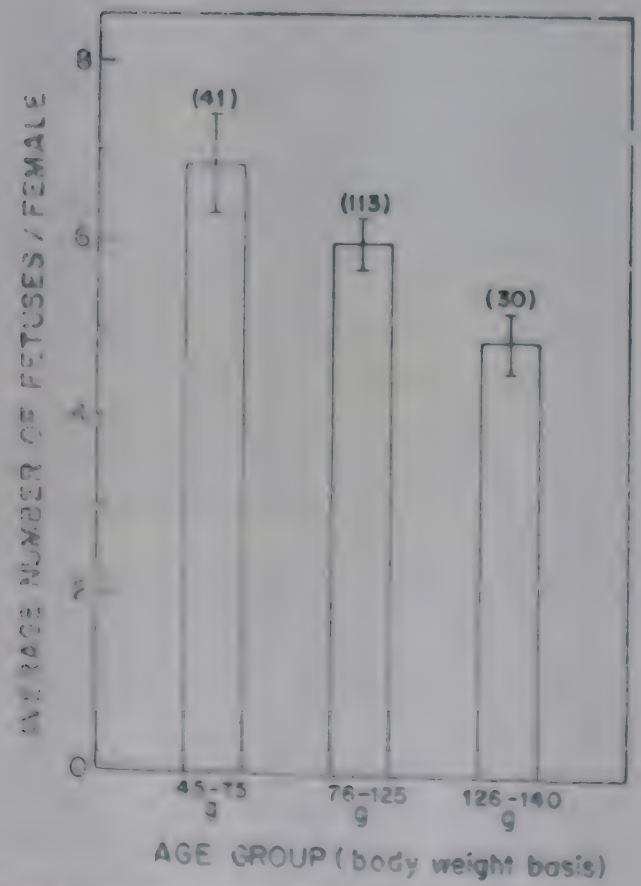


Figure 2 The average number of fetuses in relation to the weight group of the mother. The vertical lines show SEM and the figures in parenthesis indicate the number of pregnant females

active folliculogenesis. Such ovaries contained fully formed Graafian follicles and other follicles at different stages of development. Few atretic follicles were also seen. Functional corpora lutea along with one or two degenerating ones were also observed. The ovarian stroma was less dense but well vascularized. The stromal cells were large and polyhedral with distinct boundaries; the nuclei were densely stained, while the cytoplasm was light and vacuolated.

From December to February, ovaries showed anoestrus condition. Only partially developed follicles were observed and Graafian follicles or functional corpora lutea were absent. In some cases, few regressing corpora lutea were also observed. The number of atretic follicles showed an increase. The stroma was very dense with indistinct cells.

Number of live fetuses, corpora lutea and suckling young ones (table 2): No significant difference was observed in the average number of live fetuses in gravid females during different months which varied from 5.5 to 6.5. Similarly, no difference was seen in the average number of corpora lutea per female (range 6.0 to 8.0), though these were slightly more as compared to average number of live fetuses. Average number of suckling young rats collected from each nest occupied by a single mother ranged from 5.0 to 6.5. These values were almost same as the number of live fetuses.

Age vs. incidence of pregnancy and number of fetuses (figure 2): Distribution of pregnancies in females of different age groups showed a specific correlation. Maximum number of pregnant females belonged to middle age group (76–125g), which was more than double the number of pregnant females in the lower age group (45–75g) and three times more than in the higher age group (126–140g).

The number of fetuses also showed a relationship with the age of the mother. The average number of fetuses was maximum

Table 2 *Number of live fetuses and corpora lutea in pregnant rats and the number of young ones per nest during different months* (Mean ± SEM)

Month	Number of pregnant females	Average number of live fetuses per female	Average number of corpora lutea per female	Number of nest observed	Average number of young per nest
January	—	—	—	—	—
February	—	—	—	—	—
March	5	6.2 ± 0.58	7.0 ± 1.50	8	5.0 ± 1.00
April	9	6.0 ± 0.89	6.6 ± 0.65	15	5.5 ± 0.76
May	16	6.0 ± 1.00	7.5 ± 0.35	8	6.5 ± 0.89
June	30	6.2 ± 0.34	6 8 ± 0.76	19	6.0 ± 0 50
July	11	5.5 ± 0.50	7.5 ± 0.50	5	6.0 ± 0.41
August	25	6.3 ± 0.33	7.0 ± 1.50	4	6.0 ± 1.23
September	35	5.5 ± 0.41	8.0 ± 1.00	7	7.5 ± 0.33
October	18	5.5 ± 0.50	6.0 ± 0.50	4	5 0 ± 1.50
November	33	6.0 ± 1.26	6.7 ± 1.23	12	5.7 ± 0.65
December	12	5.8 ± 1.00	6.3 ± 1.45	17	5.8 ± 0.89

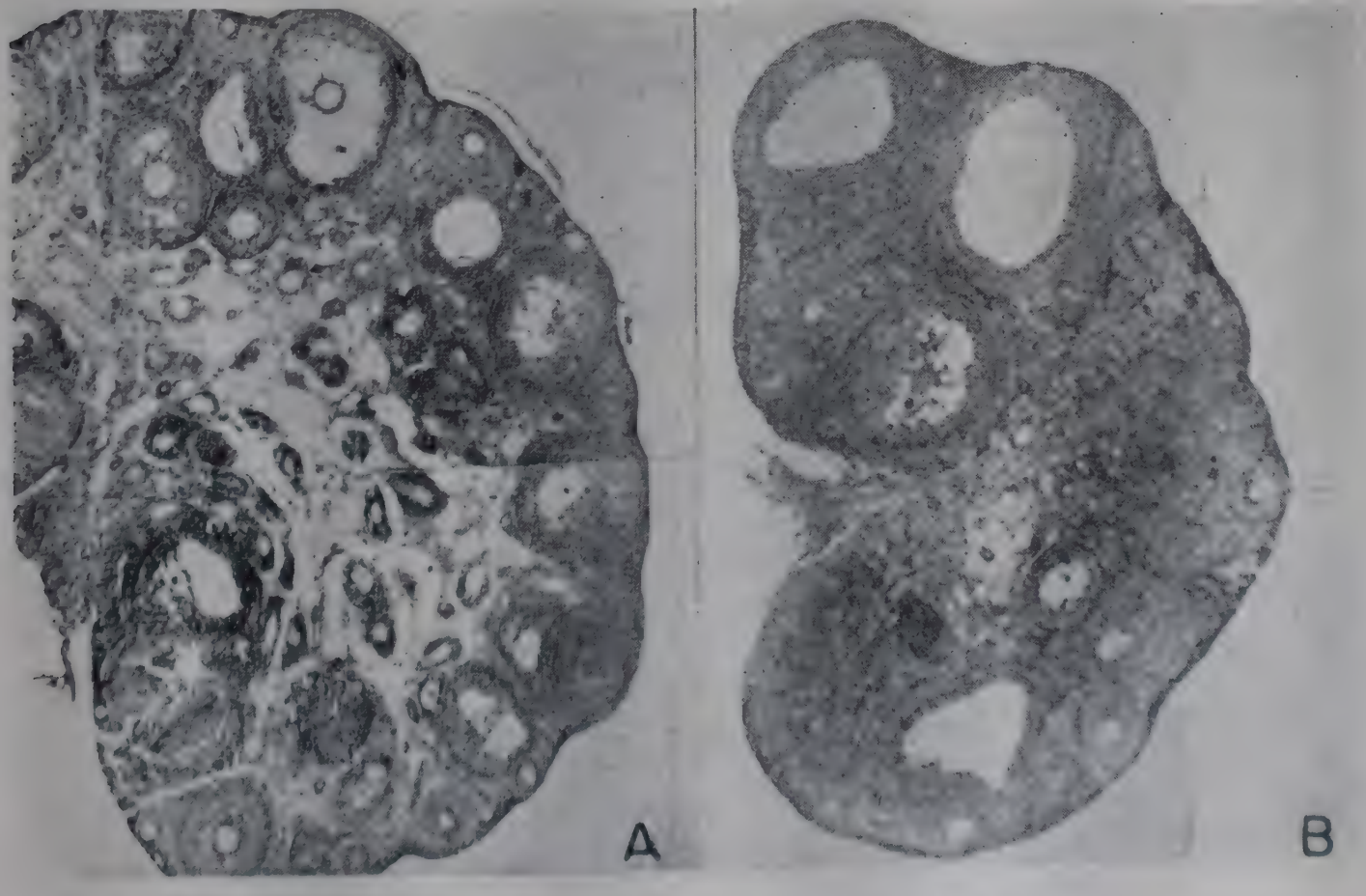


Figure 3 T S of ovary. A. breeding phase (40); B. non-breeding phase (40)

(6.87) in the lowest age group and decreased with the increase in age of the mother.

Discussion

The female field rat, *R. r. brunneusculus*, like the male, is a seasonal breeder. Monthly observations on the changes in the weight and histology of the ovary suggested that the sexual activity extends from March to November, while the sexually inactive or anoestrus phase is rather short and restricted to winter months (December to February). The breeding cycle of this field rat is comparable to that of the females of many other rodent species (Wade 1927, Foster 1934, MacMillen 1964, Horner & Taylor 1968, Srivastava 1968, Seth & Prasad 1969, Prakash 1975, Guraya & Gupta 1977) which show either identical or a shorter period of sexual activity. The ovary during the breeding and non-breeding phases in *R. r. brunneusculus* shows histology similar to those previously reported in other seasonally breeding rodents.

The monthly distribution of subadult, adult non-pregnant, pregnant and lactating females further provides support for seasonal breeding activity in this species of the field rat. The presence of relatively high number of pregnant females in June and August indicates peak breeding activity during these months. Such peaks in the breeding activity have also been reported for other rodents like the Indian desert gerbil, *Meriones hurrianae* (Prakash 1964, Kaul & Ramaswamy 1969) and north Indian gerbil, *Tatera indica indica* (Jain 1970). The non-availability of pregnant and lactating females in January clearly shows absence of breeding activity which is consistent with the occurrence of non-breeding phase during this and subsequent one or two months. However, the occurrence of a few pregnant females during February and March, when the majority of the animals are sexually inactive, is not of much significance and may be due to the premature onset of breeding in some animals.

Occurrence of lactating females from April to December with a peak in July and September is probably due to high rate of littering during these months; this seems to be reasonable since a high percentage of pregnant females was observed in the months preceding to these peaks. The non-pregnant adult females occur throughout the year but are more numerous during January and March. The number of these animals from July to September is comparatively low which may be due to the fact that large number of mature females are either pregnant or lactating at this time.

The subadult females are found throughout the year but their significant increase from April to January and then fall in number during February and March confirms that this species is a seasonal breeder. The subadults which are found in February and March may be the result of stray cases of deliveries in these or in previous months. The significant increase in the percentage of subadults from September to December indicates intense breeding a few months after the onset of the actual breeding phase.

In contrast to the situation prevailing in some seasonally breeding temperate and tropical animals, especially birds, the role of any particular environmental factor in regulating the reproductive cycle is not evident. Low temperature or short photoperiod during winter months (December to February) could possibly be detrimental or a blocking factor to breeding activity. Everett (1972) also reported that in winter months many female rats become infertile. Besides environmental factors, the availability of food may also have some regulatory effect on the breeding cycle. Animals are normally sexually active at the time when the food in some or other form is readily available in the fields. The non-breeding phase generally coincides with the period when the fields are barren and there

is an overall scarcity of food. However, in the absence of any concrete evidence it is rather difficult to implicate any factor(s) in the regulation of the annual reproductive cycle of this rat species.

The average number of live fetuses (5.5 to 6.5) in pregnant females during different months did not show any significant difference though individual variations were noticeable in the number of fetuses which varied from 2 to 10 fetuses per female. This is similar to the reports in other species of rats and mice (Brambell 1956, Taylor 1961, Asdell 1964); however, in some rodent species, even the monthly variations in the average fetus count have been reported (Prasad 1961, Kaul & Ramaswamy 1969). The individual variations in the number of fetuses seem to be related with the age. With the increase in age there is corresponding decrease in the number of fetuses indicating gradual decrease in productivity. However, the maximum number of pregnant females belong to middle age group which suggests that in contrast to fetal count, the breeding activity increases with age upto a certain limit and then declines. An interesting observation was that the average number of live fetuses is very close to the average number of functional corpora lutea or young ones collected from each nest occupied by a single female. This clearly indicates an extremely low rate of pre- or post-natal mortality in contrast to that reported in some species of the family Cricetidae (Beer et al. 1957, Prasad 1961).

Acknowledgements

The authors thank the Government of Mizoram and the local people, especially the farmers for their co-operation and providing the necessary facilities for carrying out this work in different areas of Mizoram. The work was supported by a grant from the Indian Council of Agricultural Research, New Delhi, which is gratefully acknowledged.

Refcrences

Asdell S A 1964 *Patterns of Mammalian Reproduction* (2nd edn) pp 213–409 (London: Constable and Co. Ltd.)

Beer J E, Macleod C R and Frenzel L D 1957 Prenatal survival and loss in some cricetid rodents; *J. Mammal.* **38** 392–402

Brambell F W R 1956 Ovarian changes; in *Marshall's Physiology of Reproduction* (3rd edn) Vol. I Part I pp 397–542 ed. A S Parkes (London: Longmans, Green and Co Ltd.)

Chauhan N S and Saxena R N 1985 Annual reproductive cycle of the male field rat, *Rattus rattus brunneusculus* (Hodgson) in hilly terrain of Mizoram; *J. Bombay Nat. Hist. Soc.* (in press)

Everett J W 1972 Brain pituitary gland and the ovarian cycle; *Biol. Reprod.* **6** 3–12

Foster M A 1934 The reproductive cycle in the female ground squirrel, *Citellus tridecemlineatus* (Mitchill); *Amer. J. Anat.* **54** 487–511

Guraya S S and Gupta S 1977 Observations on the reproductive biology of the soft-furred rat, *Millardia meltada; Proc. All India Rodent Sem.*, Ahmedabad pp 70–75

Horner B E and Taylor J M 1968 Growth and reproductive behavior in the southern grasshopper mouse; *J. Mammal.* **49** 644–660

Jain A P 1970 Body weights, sex ratio, age structure and some aspects of reproduction in the Indian gerbil, *Tatera indica indica* (Hardwicke) in the Rajasthan desert, India; *Mammalia* **34** 415–432

Kaul D K and Ramaswamy L S 1969 Reproduction in the Indian desert gerbil, *Meriones hurrianae* (Jerdon); *Acta Zoologica* **50** 233–248

MacMillen R E 1964 Population ecology, water relations and social behaviour of a southern California semi-desert rodent fauna; *Univ. Calif. Publ. Zool.* **71** 1–59

Prakash I 1964 Eco-toxicology and control of Indian desert gerbil, *Meriones hurrianae* (Jerdon). Part II. Breeding season, litter size and post-natal development; *J. Bombay Nat. Hist. Soc.* **61** 142–149

—— 1975 The population ecology of the rodents of the Rajasthan desert, India; in *Rodent in Desert Environment* pp 75–186 eds. I Prakash and PK Ghosh (The Hague: Dr W Junk b.v. Verlag Publishers)

Prasad M R N 1961 Reproduction in the female Indian gerbille, *Tatera indica cuvierii* (Waterhouse); *Acta Zoologica* **32** 245–256

Seth P and Prasad M R N 1969 Reproductive cycle of the female five-striped Indian palm squirrel, *Funambulus pennanti* (Wroughton); *J. Reprod. Fertil.* **20** 211–222

Srivastava A S 1968 *Rodent Control for Increased Food Production*; 1–152 (Kanpur: Rotary Club (west)),

Taylor J M 1961 Reproductive biology of the Australian bush rat, *Rattus assimilis; Univ. Calif. Publ. Zool.* **60** 1–66

Wade O 1927 Breeding habits and early life of the thirteen-striped ground squirrel, *Citellus tridecemlineatus* (Mitchill); *J. Mammal.* **8** 269–276

Proc. Indian natn. Sci. Acad. B51 No. 5 pp 566-573 (1985)

Phagostimulants in Artificial Feeding Systems of Rat Fleas *Xenopsylla cheopis* and *X. astia*

S GEETHA DEVI and R S PRASAD

Department of Zoology, University of Kerala, Karyavattom, Trivandrum 695581

(Received 20 May 1985)

Four different nucleotides ATP, ADP, AMP and cAMP were tested singly and in combination. When tested singly at three different concentrations only ATP was found to be the effective phagostimulant. Of the six combinations tested only three which include ATP were found to be phagostimulatory. No synergistic effect was noticed with each other.

Key Words: Nucleotides, Phagostimulant, Synergistic effect

Introduction

Hosoi (1958) observed adenosine-5-phosphate in blood stimulating feeding in the mosquito *Culex pipiens*. Subsequently adenine nucleotides were proved to be phagostimulants in the mosquito *Aedes aegypti* (Galun et al. 1963, Galun 1967), in the rat flea *Xenopsylla cheopis* (Galun 1966), in the tick *Ornithodoros tholozani* (Galun & Kindler 1968), in the tsetse fly *Glossina austeni* (Galun & Margalit 1969), in *Rhodnius prolixus* (Friend 1965, Friend & Smith 1971) and in *Simulium venustum* (Sutcliffe & McIver 1979). Several other nucleotides were also found phagostimulatory, e.g. guanosine triphosphate (GTP), cytosine triphosphate (CTP) and uridine triphosphate (UTP) in *R. prolixus* (Friend 1965), diphosphopyridine nucleotide (DPN) in *A. aegypti* (Galun 1967), inosine triphosphate (ITP) and GTP in *O. tholozani* (Galun & Kindler 1968). According to Galun and Rice (1971) the source of ATP is platelets rather than RBC. However, according to Smith (1979) the source of gorging stimulant in the case of *Rhodnius prolixus* is RBC.

In the present study an attempt is made to observe the phagostimulatory effect of certain nucleotides singly and in combination.

Materials and Methods

Seven day old fleas (fleas fed for 5 days and starved for 2 days) were used for the experiment.

In vitro Feeding

The technique used for artificial feeding was that described by Kartman (1958). The skin from the abdominal region of a one-month-old white rat *Rattus norvegicus* albino was used as the feeding membrane. Diet

was maintained at 36–38°C in a 50 ml beaker. Blood for artificial feeding was collected from *R. norvegicus* albino by cardiac puncture (Gay 1965). Fresh blood collected each day was used for feeding. Heparin (0.2mg/ml) was used as anticoagulant. Blood plasma was collected by centrifuging the heparinized whole blood at 3000 rpm for 10 min.

Phagostimulatory properties of ATP, ADP, AMP and cAMP were tested singly and in combination using the following techniques. The required quantity of the necleotide(s) to be tested was dissolved in normal saline and 0.1ml of this solution was added to 1ml of plasma. In the former experiments (nucleotides used singly) the concentration was adjusted in such a way that when 0.1ml of the prepared solution was added to 1ml of plasma the concentration of the substance in plasma would be 0.004M. The experiment was repeated using two other concentrations–0.008M and 0.002M. Comparisons were made with control diet consisting of plasma without nucleotides.

Two sets of experiments were conducted as far as the latter (combination) was concerned. In the first case three combinations were tried—ATP+cAMP; ATP+AMP; ATP+ADP. The concentration of each nucleotide was adjusted to be 0.004M as described above. In this experiment comparisons were made with two types of solutions one consisted of a mixture of plasma and 0.004M ATP and the other plasma without adenosine triphosphate (controls).

In the second case three other combinations were tried ADP+cAMP; AMP+cAMP and ADP+AMP. Controls were same as the above.

The fleas were weighed using a micro analytical balance. The weight of blood ingested was calculated from the difference between weights of the fleas before and after feeding.

Results

The results summarised in tables 1–5 clearly indicate that ATP is the only nucleotide which showed phagostimulatory property. None of the nucleotides showed synergistic action with each other.

Discussion

Several experiments done on haematophagous arthropods like *Aedes aegypti, Culex pipiens, Glossina austeni, Rhodnius prolixus, Xenopsylla cheopis* and *Simulium venustum* proved that nucleotides as such, as well as in combination with other nucleotides act as phagostimulants. Galun et al. (1963) arranged the different nucleotides according to their increasing efficiency as AMP, ADP, ATP and A-tetra-P. She later suggested (Galun 1967) the importance of location and number of phosphate groups as important aspects in conferring phagostimulatory property to the nucleotides. Subsequently Galun and Kindler (1968) observed ATP, ITP, and GTP to be phagostimulatory in the presence of glucose in the tick *Ornithodoros tholozani.* According to Friend (1965) nucleotides having high energy phosphate bonds like ATP, GTP, CTP, UTP as well as other compounds like riboflavin-5-phosphate, adenosine-5-monophosphate, and adenosine 3'-5'-monophosphate which lack high energy phosphate bonds also stimulate feeding in *Rhodnius prolixus.* The potency of analogues of ATP declined as the difference between their molecular structure and that of ATP increased (Friend & Smith 1971, Smith & Friend 1976a). Friend and Smith (1982) showed that molecules with terminal phosphate groups were the only potent phagostimulants for *R. prolixus.* They noted certain non-nucleotide phosphate compounds such as pyrophosphate and methylene diphosphonic acid also as potent phagostimulants in *R. prolixus.* According to them single phosphate ions aslo have some phagostimulatory property. However, the black fly *Simulium venustum* was not sensitive to reduction in the phosphate chains of ATP; in the case of this insect

Table 1 *Role of nucleotides (0.004 M) on feeding*•

Nucleotide tested	*X. astia*		*X. cheopis*	
	Newly emerged unfed females	7-day-old females	Newly emerged unfed females	7-day-old females
1. ATP	0.533 (SE±0.027)	1.300 (SE±0.047)	0.467 (SE±0.027)	0.900 (SE±00.047)
2. ADP	0.233 (SE±0.054)	0.367 (SE±0.098)	0.067 (SE±0.054)	0.033 (SE±0.027)
3. AMP	0.200 (SE±0.000)	0.467 (SE±0.098)	0.033 (SE±0.027)	0.067 (SE±0.027)
4. CAMP	0.267 (SE±0.027)	0.367 (SE±0.098)	0.067 (SE±0.054)	0.10 (SE±0.047)
5. Plasma (Control)	0.233 (SE±0.027)	0.367 (SE±0.054)	0.067 (SE±0.027)	0.100 (SE±0.047)

RESULTS OF *t*-TEST

Comparisons				
1 and 2	4.02 (S)	7.00 (S)	5.37 (S)	13.00 (S)
1 and 3	10.00 (S)	6.25 (S)	9.19 (S)	12.50 (S)
1 and 4	5.66 (S)	7.00 (S)	5.37 (S)	9.80 (S)
1 and 5	6.36 (S)	10.58 (S)	8.48 (S)	9.80 (S)
2 and 3	0.50 (NS)	0.59 (NS)	0.45 (NS)	0.71 (NS)
2 and 4	0.45 (NS)	0.00 (NS)	0.00 (NS)	1.00 (NS)
2 and 5	0.00 (NS)	0.00 (NS)	0.00 (NS)	1.00 (NS)
3 and 4	2.00 (NS)	0.50 (NS)	0.45 (NS)	0.50 (NS)
3 and 5	1.00 (NS)	0.73 (NS)	0.71 (NS)	0.50 (NS)
4 and 5	0.71 (NS)	0.00 (NS)	0.00 (NS)	0.00 (NS)

• Values given relate to milligrams of bloodingested per 10 fleas and average of 3 replicates of 10 fleas each
S. Significant at 5% level
NS. Not Significant

Table 2 *Role of nucleotides (0.008M) on feeding**

Nucleotide tested	*X. astia*		*X. cheopis*	
	Newly emerged unfed females	7-day-old females	Newly emerged unfed females	7-day-old females
1. ATP	0.500 (SE±0.047)	1.167 (SE±0.072)	0.467 (SE±0.027)	0.933 (SE±0.072)
2. ADP	0.100 (SE±0.047)	0.267 (SE±0.027)	0.033 (SE±0.027)	0.200 (SE±0.047)
3. AMP	0.100 (SE±0.047)	0.233 (SE±0.027)	0.100 (SE±0.047)	0.100 (SE±0.047)
4. CAMP	0.067 (SE±0.027)	0.100 (SE±0.082)	0.100 (SE±0.047)	0.200 (SE±0.047)
5. Plasma (Control)	0.067 (SE±0.054)	0.100 (SE±0.047)	0.067 (SE±0.027)	0.167 (SE±0.072)

RESULTS OF *t*-TEST

Comparisons				
1 and 2	4.90 (S)	9.55 (S)	9.19 (S)	6.96(S)
1 and 3	4.90 (S)	9.90 (S)	5.50 (S)	7.91 (S)
1 and 4	6.50 (S)	8.00 (S)	5.50 (S)	6.96 (S)
1 and 5	4.91 (S)	10.12 (S)	8.49 (S)	6.15 (S)
2 and 3	0.00 (NS)	0.71 (NS)	1.00 (NS)	1.22 (NS)
2 and 4	0.50 (NS)	1.58 (NS)	1.00 (NS)	0.00 (NS)
2 and 5	0.38 (NS)	2.50 (NS)	0.71 (NS)	0.32 (NS)
3 and 4	0.50 (NS)	1.26 (NS)	0.00 (NS)	1.22 (NS)
3 and 5	0.38 (NS)	2.00 (NS)	0.50 (NS)	0.63 (NS)
4 and 5	0.00 (NS)	0.00 (NS)	0.50 (NS)	0.32 (NS)

* Values given relate to milligrams of blood ingested per 10 fleas and average of 3 replicates of 10 fleas each
S, Significant at 5% level
NS, Not significant

Table 3 *Role of nucleotides (0.002M) on feeding**

Nucleotide tested	*X. astia*		*X. cheopis*	
	Newly emerged unfed females	7-day-old females	Newly emerged unfed females	7-day-old females
1. ATP	0.500 (SE±0.047)	1.333 (SE±0.027)	0.433 (SE±0.027)	1.133 (SE±0.072)
2. ADP	0.067 (SE±0.027)	0.300 (SE±0.094)	0.067 (SE±0.027)	0.267 (SE±0.119)
3. AMP	0.133 (SE±0.027)	0.167 (SE±0.098)	0.100 (SE±0.047)	0.167 (SE±0.054)
4. cAMP	0.067 (SE±0.054)	0.233 (SE±0.109)	0.100 (SE±0.047)	0.100 (SE±0.047)
5. Plasma (Control)	0.100 (SE±0.047)	0.233 (SE±0.119)	0.067 (SE±0.054)	0.100 (SE±0.047)

RESULTS OF *t*-TEST

Comparisons				
1 and 2	6.50 (S)	8.62 (S)	7.78 (S)	5.10 (S)
1 and 3	5.50 (S)	9.35 (S)	5.00 (S)	8.74 (S)
1 and 4	4.91 (S)	7.99 (S)	5.00 (S)	9.80 (S)
1 and 5	4.90 (S)	7.38 (S)	4.92 (S)	9.80 (S)
2 and 3	1.41 (NS)	0.80 (NS)	0.50 (NS)	0.63 (NS)
2 and 4	0.00 (NS)	0.38 (NS)	0.50 (NS)	1.07 (NS)
2 and 5	0.50 (NS)	0.36 (NS)	0.00 (NS)	1.07 (NS)
3 and 4	0.89 (NS)	0.37 (NS)	0.00 (NS)	0.76 (NS)
3 and 5	0.50 (NS)	0.35 (NS)	0.38 (NS)	0.76 (NS)
4 and 5	0.38 (NS)	0.00 (NS)	0.38 (NS)	0.00 (NS)

* Values given relate to milligrams of blood ingested per 10 fleas and average of 3 replicates of 10 fleas each
S, Significant at 5% level
NS, Not significant

Table 4 *Role of different combination of nucleotides on feeding**

Nucleotide tested	*X. astia*		*X. cheopis*	
	Newly emerged unfed females	7-day-old females	Newly emerged unfed females	7-day-old females
1. ATP	0.833 (SE±0.027)	1.467 (SE±0.027)	0.733 (SE±0.027)	1.267 (SE±0.027)
2. ATP+ADP	0.833 (SE±0.054)	1.467 (SE±0.027)	0.767 (SE±0.027)	1.167 (SE±0.027)
3. ATP+AMP	0.800 (SE±0.047)	1.400 (SE±0.047)	0.700 (SE±0.047)	1.233 (SE±0.027)
4. ATP+cAMP	0.833 (SE±0.027)	1.433 (SE±0.027)	0.667 (SE±0.027)	1.200 (SE±0.047)
5. Plasma (Control)	0.200 (SE±0.047)	0.600 (SE±0.047)	0.167 (SE±0.027)	0.533 (SE±0.027)

RESULTS OF *t*-TEST

Comparisons				
1 and 2	0.00 (NS)	0.00 (NS)	0.71 (NS)	2.12 (NS)
1 and 3	0.50 (NS)	1.00 (NS)	0.50 (NS)	0.71 (NS)
1 and 4	0.00 (NS)	0.71 (NS)	1.41 (NS)	1.00 (NS)
1 and 5	9.50 (S)	13.00 (S)	12.02 (S)	15.56 (S)
2 and 3	0.38 (NS)	1.00 (NS)	1.00 (NS)	1.41 (NS)
2 and 4	0.00 (NS)	0.71 (NS)	2.12 (NS)	0.50 (NS)
2 and 5	7.18 (S)	13.00 (S)	12.73 (S)	13.44 (S)
3 and 4	0.50 (NS)	0.50 (NS)	0.50 (NS)	0.50 (NS)
3 and 5	7.35 (S)	9.80 (S)	8.00 (S)	14.84 (S)
4 and 5	9.50 (S)	12.50 (S)	10.61 (S)	10.00 (S)

* Values given relate to milligrams of blood ingested per 10 fleas and average of 3 replicates of 10 fleas each
S, Significant at 5% level
NS, Not Significant

Table 5 *Role of different combinations of nucleotides on feeding**

Nucleotides tested	*X. astia*		*D. cheopis*	
	Newly emerged unfed females	7-day-old females	Newly emerged unfed females	7-day-old females
1. ATP	0.733 (SE±0.027)	1.433 (SE±0.027)	0.667 (SE±0.027)	1.333 (SE±0.027)
2. ADP+cAMP	0.233 (SE+0.054)	0.700 (SE±0.047)	0.233 (SE±0.027)	0.433 (SE±0.027)
3. AMP+cAMP	0.267 (SE±0.054)	0.600 (SE±0.047)	0.167 (SE±0.027)	0.533 (SE±0.072)
4. ADP+AMP	0.233 (SE±0.027)	0.633 (SE±0.027)	0.133 (SE±0.027)	0.533 (SE±0.027)
5. Plasma (Control)	0.266 (SE±0.027)	0.633 (SE±0.027)	0.200 (SE±0.047)	0.533 (SE±0.027)

RESULTS OF *t*-TEST

Comparisons				
1 and 2	6.71 (S)	11.00 (S)	9.19 (S)	19.09 (S)
1 and 3	6.26 (S)	12.50 (S)	10.61 (S)	8.49 (S)
1 and 4	10.61 (S)	16.97 (S)	11.31 (S)	16.97 (S)
1 and 5	9.90 (S)	16.97 (S)	7.00 (S)	16.97 (S)
2 and 3	0.35 (NS)	1.22 (NS)	1.41 (NS)	1.05 (NS)
2 and 4	0.00 (NS)	1.00 (NS)	2.12 (NS)	2.12 (NS)
2 and 5	0.45 (NS)	1.00 (NS)	0.50 (NS)	2.12 (NS)
3 and 4	0.45 (NS)	0.50 (NS)	0.71 (NS)	0.00 (NS)
3 and 5	0.00 (NS)	0.50 (NS)	0.50 (NS)	0.00 (NS)
4 and 5	0.71 (NS)	0.00 (NS)	1.00 (NS)	0.00 (NS)

* Values given relate to milligrams of blood ingested per 10 fleas and average of 3 replicates of 10 fleas each
S, Significant at 5% level
NS, Not Significant

ADP was more potent in stimulating gorging than ATP; AMP and adenine were also highly stimulatory (Sutcliffe & McIver 1979, Smith & Friend 1982). Smith and Friend (1976b) showed that a mixture of several nucleotides in a combination has certain synergistic effects in feeding.

It is evident from the present work that the only nucleotide with phagostimulatory property, as far as rat flea is concerned, is ATP. This observation is in confirmity with that of Galun (1966). In contrast with the observation in *Rhodnius* by Friend and Smith (1976b), it has been noted that combination of the nucleotides has no synergistic effect in these fleas.

References

Friend W G 1965 The gorging response in *Rhodnius prolixus* Stahl; *Can. J. Zool.* **43** 125–132

—— and Smith J J B 1971 Feeding in *Rhodnius prolixus*; Potencies of nucleotide phosphates in initiating gorging; *J. Insect Physiol.* **17** 1315–1320

—— and —— 1982 ATP analogues and other phosphate compounds as gorging stimulants for *Rhodnius prolixus*; *J. Insect Physiol.* **28** 371–376

Galun R 1966 Feeding stimulants of the rat flea *Xenopsylla cheopis* Roth; *Life Sci.* **5** 1335–1342

—— 1967 Feeding stimuli and artificial feeding; *Bull. Wld. Hlth. Org.* **36** 590–593

——, Avi-Dor Y, Bar-Zeev M 1963 Feeding response in *Aedes aegypti*: stimulation by adenosine triphosphate; *Science* **142** 1674–1675

—— and Kindler S H 1968 Chemical basis of feeding in the tick *Ornithodoros tholozani*; *J. Insect Physiol.* **14** 1409–1421

—— and Margalit J 1969 Adenine nucleotides as feeding stimulants of the tsetse fly *Glossina austeni* Newst.; *Nature Lond.* **22** 583–584

—— and Rice M J 1971 Role of platelets in haematophagy; *Nature, New Biol.* **233** 110–111

Gay W L 1965 *Methods of Animal Experimentation. Vol. I.* (New York: Academic Press) 382 pp

Hosoi T 1958 Adenosine 5'-phosphate as the stimulatory agent in blood for inducing gorging of the mosquito; *Nature Lond.* **181** 1664–1665

Kartman L 1954 Studies of *Pasteurella pestis* in fleas. 1. An apparatus for the experimental feeding of fleas; *Exptl. Parasitol.* **3** 525–537

Smith J J B 1979 The feeding response of *Rhodnius prolixus* to blood fractions and the role of ATP; *J. Exp. Biol.* **78** 225–232

—— and Friend W G 1976a Further studies on potencies of nucleotides as gorging stimuli during feeding in *Rhodnius prolixus*; *J. Insect Physiol.* **22** 607–611

—— and —— 1976b Potencies of combined doses of nucleotides as gorging stimulants for *Rhodnius prolixus*; *J. Insect Physiol.* **22** 1049–1052

—— and —— 1982a Diphosphoglycerate and phytic acid as feeding stimulants for the blood feeding bug, *Rhodnius prolixus*; *Comp. Biochem. Physiol.* **72A** 133–136

Sutcliffe J F and McIver S B 1979 Experiments on biting and gorging behaviour in the blackfly, *Simulium venustum*; *Physiol. Ent.* **4** 393–400

Proc. Indian natn. Sci. Acad. B51 No 5 pp 574–580 (1985)

Pollination Potential, Population Dynamics and Dispersal of Thrips Species (Thysanoptera: Insecta) Infesting Flowers of *Dolichos lablab* L. (Fabaceae)

R VELAYUDHAN, K GOPINATHAN and N BAKTHAVATSALAM
Entomology Research Institute, Loyola College, Madras 600034

(Received 6 September 1985)

Thrips flower association in *Dolichos lablab* has been discussed with special reference to species composition, pollinator dispersal, pollen stigma interaction, nectar exudation and pollen transfer mechanics.

Key Words: Thrips, Pollination, *Dolichos lablab*

Introduction

The adaptive significance of thrips-flower association in terms of pollination potential mainly depends on the dynamics of pollen transfer mechanisms. The availability of pollen-nectar as food, pollen-stigma interactions, the architecture of the pollen wall combined with the mechanisms for pollen attachment on the body of the pollinator, synchronisation of anthesis and nectar availability with the development of the pollinator as well as behavioural aspects of thrips play an essential role in pollination (Ananthakrishnan 1982). While these aspects have been highlighted with special reference to thrips-compositae interaction (Ananthakrishnan et al. 1981, Gopinathan et al. 1981, Varatharajan et al. 1983, Gopinathan & Varatharajan 1983), information on the thrips pollination in Fabaceae relates only to *Phaseolus lunatus* (Mackie & Smith 1935, Allard 1954, Lewi 1973) and on *Cajanus cajan* (Williams 1977). The nature of the thrips flower association in *Dolichos lablab* (Fabaceae) is discussed in terms of species composition, ecological succession, pollinator dispersal, pollen-stigma interaction, mode of nectar exudation and pollen transfer mechanics.

Materials and Methods

Random weekly collections of thrips were made from 20 flowers to record not only the fluctuations in the populations but also to observe the pollen load by counting the number of pollen grains attached to the various parts of the body. Dispersal of thrips from flower to flower was observed by tagging them with gilt powder. In order to assess the role of thrips in the transfer of pollen from flower to flower in an

inflorescence, emasculation technique was carried out by removing the anthers in all the buds in the upper part of the inflorescence thereby preventing self-fertilisation, at the same time covering the complete inflorescence with a plastic bag, so as to prevent other agents from pollinating the flower, thus allowing only thrips to do the role. A comparative assessment of the efficiency of pollination by various agents was estimated in terms of pod setting. This was investigated by three sets of experiments: (*a*) hand pollination, (*b*) pollination by thrips alone, and (*c*) natural condition where all the other foraging insects played their role. Aspects regarding the biology of thrips were studied by keeping cultures of thrips in flowers/ inflorescence within glass chimneys dipped in Knop's solution. Scanning electron microscopic studies to depict the mode of attachment of pollen grains on the various parts of the body were made with etherised specimens carrying pollen grains and dried in a critical point dryer. The specimens were later fixed on an aluminium stub using double adhesive tape and was coated with gold for three minutes in an ion coater. Micrographs were taken using Hitachi scanning electron microscope (Model S 415A) under 15 Kv emission current.

Observations

Megalurothrips distalis Karny and *Franklinella schultzei* the primary vector species occurred were recorded in larger frequencies, while *Haplothrips gowdeyi* and *Scirtothrips dorsalis* occurred in low frequencies within the flowers of *Dolichos lablab*. The flowering periodicity and the life-cycle of thrips synchronised with the flowering phenology, in which the blooming of *Dolichos lablab* commenced in the early part of October and continued till May, the raceme containing 8–16 flowers at various stages of development, with each flower lasting for 10–18 days. Anthesis occurred between the fifth and ninth day after the flower formation, the nectar secretion commenced from the seventh to twelfth day, the stigmatic receptivity occurred between the eight and twelfth day. The duration of the life-cycle of the pollinator was around 12–18 days on an average, the initial infestation of the flower by *M. distalis* occurred between the third and fifth day of the bud stage, with the adults feeding on the fleshy regions of the standard and keel petals as revealed by the numerous necrosed spots within the bud. Oviposition sites were mainly around the keel petals and after an incubation period of 4–5 days, the larval emergence coincided with the maximum amount of floral nectar, the feeding being mainly on the nectar and pollen. With the flower shedding, older larvae moved towards the soil for pupation and the adults emerged after three days to reinfest a new inflorescence.

While *M. distalis* and *F. schultzei* occurred continuously during the flowering period, *Haplothrips gowdeyi* was observed from January to April end and *Scirtothrips dorsalis* only in April. Temperature and rainfall had a significant impact not only on the numerical strength of thrips, but also on their reproductive biology especially the rate of fecundity and soil pupation. High temperature and excess rainfall caused a dramatic decline in the population during the months of May-June and November-December respectively (figure 1). Population trends also revealed that the weeds and other crop plants in the same vicinity played an important role in the sustenance of the pollinator population during the offseason of the crop. Figure 2 highlights the disposal of thrips species during the course of the year, *Dolichos lablab* blooming between October to June with the peak of flowering period being between December and early March, the population build-up of thrips also occurring during the same period. This

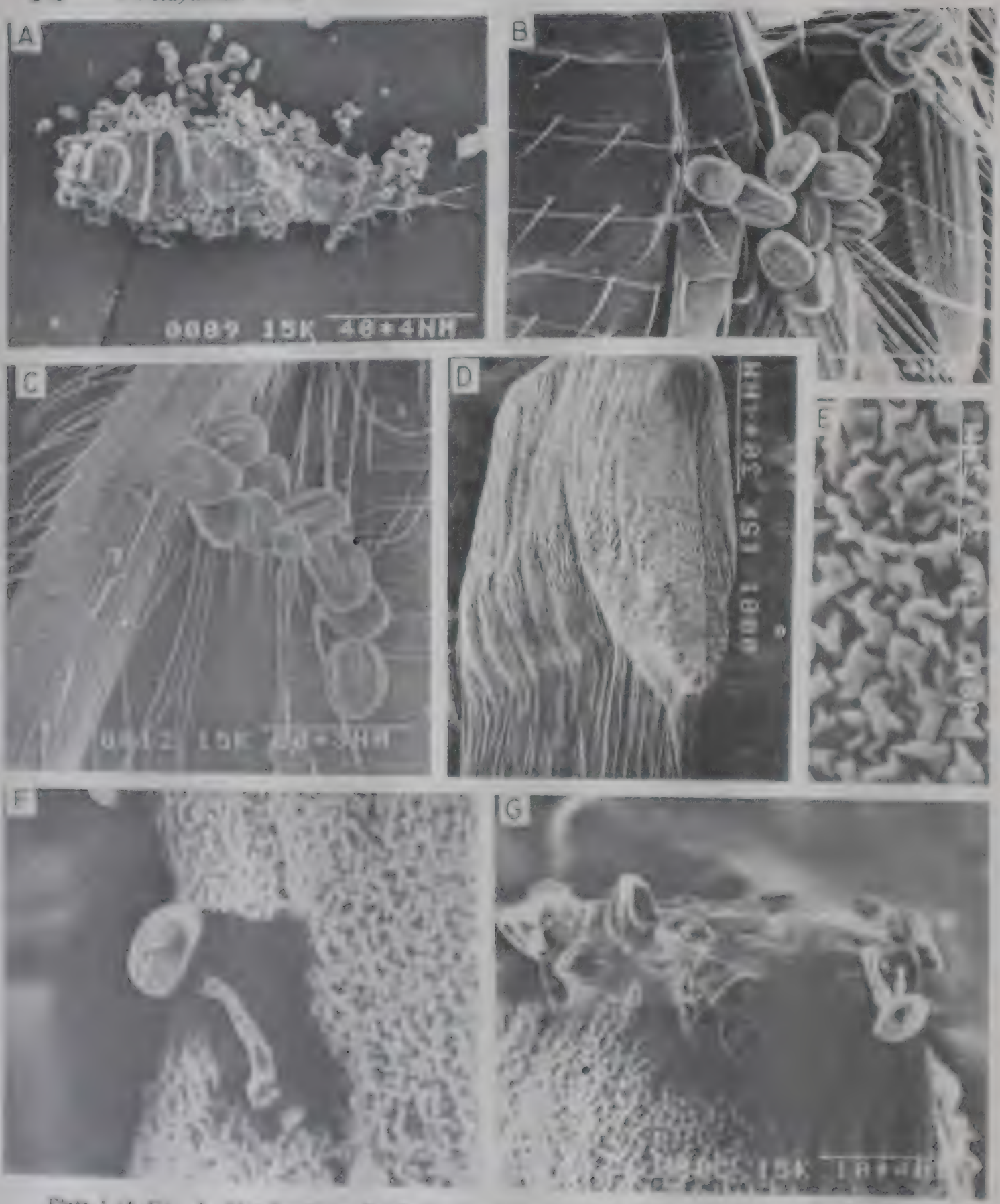


Plate I (A–G). A. *Megalurothrips distalis* larvae with pollen load on its body; B. *Frankliniella schultzei* with pollen load on its wings; C. *Megalurothrips distalis* with pollen load on its wings; D. Corrugated stigma of *Dolichos lablab* showing corrugated receptive area; E. Receptive area showing elongated cuticle; F & G. Disappearance of corrugated receptive area on the stigmatic surface on pollen germination

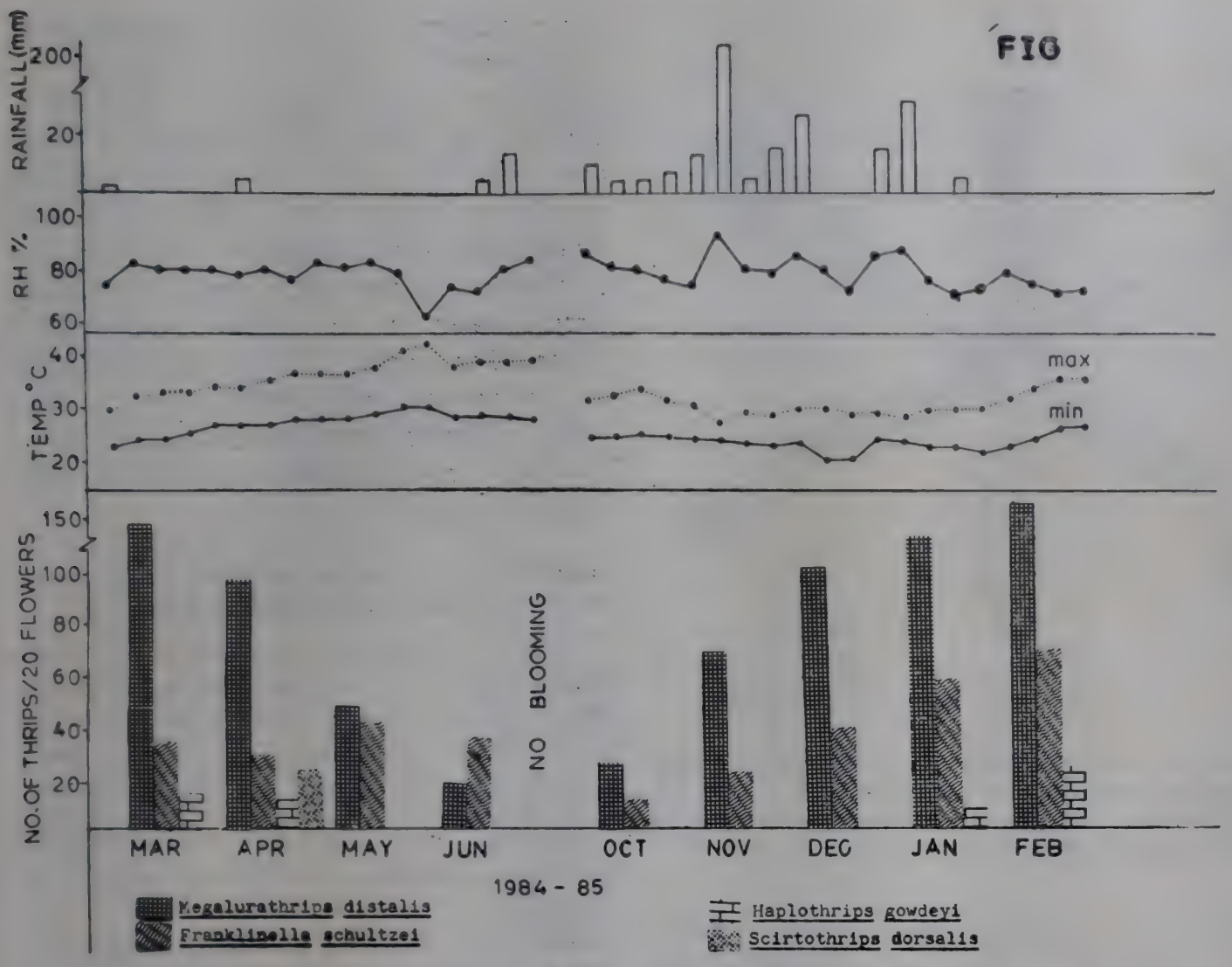


Figure 1 Population dynamics of the thrips species inhabiting *Dolichos lablab*

increase in the population seems to be due to the dispersal of thrips between crops like *Solanum melogena*, *Cajanus cajan* and *Sesbania aegyptiaca* which incidentally harbour the same species of thrips and also blooms during the same period. The figure also clearly depicts the occurrence of the thrips species on *Pongamia glabra* flowers and also the flowers of *Vigna catjang* during the offseason of the crop and incidentally coincides with the blooming period of the latter. Weeds like *Tridax procumbens* (Compositae), *Ruellia tuberosa* (Acanthaceae), *Ipomoea* sp. (Convolvulaceae) etc., serve as perennial reservoirs for thrips especially *F. schultzei*. *Megalurothrips distalis* harboured a heavier pollen load on its body than *Franklinella schultzei*, their larvae carrying more pollen than adults (plate 1A–C). Table 1 depicts a clear correlation of pollen load with the number of setae on the various parts of the body.

Scanning electron microscopic studies on the pollen as well as the receptive surface of the stigmatic tip showed the mode of attachment of the pollen on the stigmatic surface. The smooth pollen grains with three colpi on its wall (plate 1B) get attached to the corrugated receptive area of the stigma which forms a rough area for the quick adherence of the smooth pollen (plate 1D and E). As soon as the pollen grains land on the receptive area, exudation of a liquid substance occurred in the area surrounding the pollen and the developing pollen tube, and this exudate formed a suitable medium

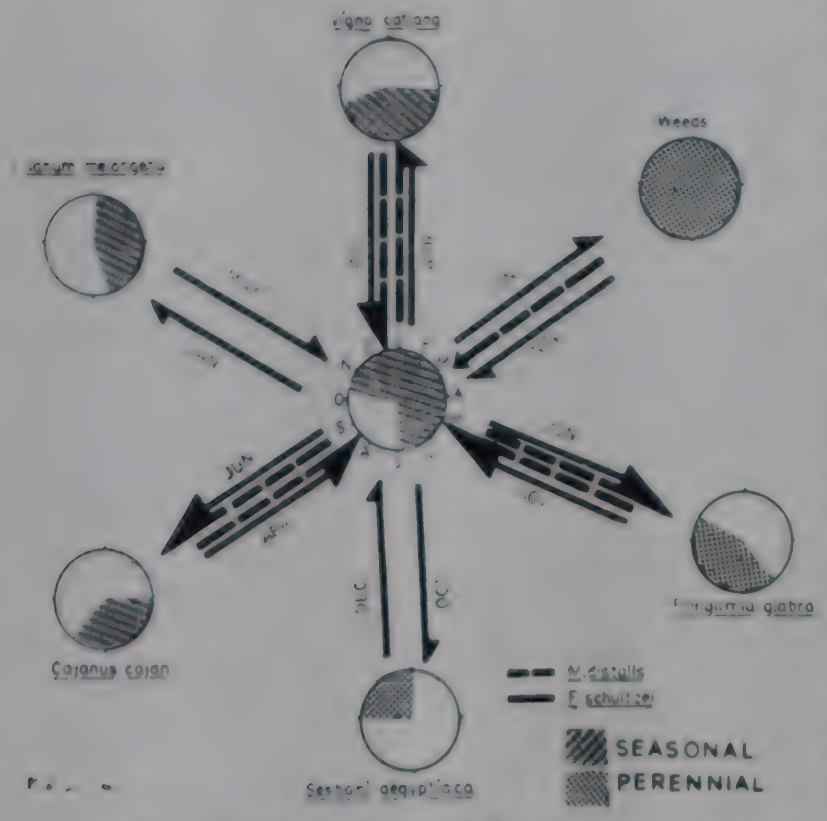


Figure 2 Dispersal pattern of thrips species among *Dolichos lablab* and other alternate hosts
(The central disc represents *Dolichos lablab* and its occurrence in different months of the year)

for pollen germination and growth. Under the influence of the viscous exudate the corrugated receptive area in the immediate vicinity of the pollen grain and the pollen tube appear disintegrated or masked. (Plate IF and G). Apart from this the pollen grain on its part exhibited a sticky nature aiding in its adherence. Pollen collected from flowers seven to eight days after anther dehiscence, showed better germination resulting in efficient seed setting. Nectar secretion commenced when the flowers were about to open, reaching its peak during anther dehiscence, and stigmatic receptivity. Numerous modified stomata were evident within the nectary with starch grains in their guard cells.

Thrips infesting the flowers at the bud stage effect early pollination. Frequent movements of thrips inside the keel petals enabled the transfer of pollen to the receptive surface of the capitate stigma, the movement resulting in considerable stress on the anther wall enchancing anther dehiscence. In order to confirm the interfloral movement of larvae and adult thrips, buds in the 2nd and 3rd row from the tip of the inflorescence were emasculated and completely covered with a polythene bag and adult thrips and larvae were released in the opened basal flowers of the inflorescence. Pod setting was evident in the emasculated flowers after 15 days thus confirming interfloral pollen movement leading to cross fertilization which is brought about by thrips.

Table 1 *Pollen carrying efficiency of* M. distalis *and* F. schultzei *Megalurothrips distalis*

Region of the body	Number of setae	Number of pollen	Correlation coefficient (value of r)
ADULT			
Head	8	6±1.167	0.9395
Thorax	24	22±1.077	0.95507
Abdomen	34	36±1.1674	0.9957
Wings	60	17±0.927	0.9194
Legs	8	11±1.000	0.9838
LARVAE			
Head	2	6±0.894	0.9600
Thorax	12	21±0.510	0.9980
Abdomen	24	38±0.8602	0.9990
Legs	6	17±1.772	0.9569
Franklinella schultzei:			
ADULT			
Head	2	2±1.092	0.9200
Thorax	8	4±0.548	0.9647
Abdomen	30	11±0.374	0.990
Wings	52	9±1.694	0.9450
Legs	14	7±1.020	0.9579
LARVAE			
Head	2	3±0.583	0.9808
Thorax	6	8±0.245	0.9808
Abdomen	22	9±0.304	0.9999
Legs	8	7±1.021	0.9578

Discussion

Synchronisation in terms of flowering periodicity and incidence of the pollinating thrips appear to be controlled by the population build-up of thrips, eventually resulting in dispersal. During the non-flowering season of *Dolichos lablab* the thrips species were forced to disperse to other weed plants in the same locality, which incidentally flowers during that period (July–September), thus enabling the species to sustain their population during the offseason of the crop. Similar type of dispersal behaviour was observed by Ananthakrishnan et al. (1981) on the flower of compositae.

The ability of thrips to carry pollen grains from the anther to the stigma of the same flower or of another flower depends on the size, viscosity, exine architecture of the pollen grains, attractiveness of the flower as well as the population, diversity, number of body setae and body surface texture of thrips species present. Pollen availability and the carrying capacity of thrips determine the pollination potential of these insect species. While *M. distalis* and *F. schultzei* played a major role in the pollination of the flowers, that of *Haplothrips gowdeyi* and *Scirtothrips dorsalis* were insignificant due to their low population and seasonal occurrence. However, in both the species the larvae were more effective in carrying a heavier load of pollen grains than the adults.

Floral nectaries occurring within the flowers are directly associated with pollination (Fahn 1982), and the ability of thrips to enter the flower at its bud stage at a time when nectar and pollen are not readily available, detection of the specialised stomata of the nectaries and the fleshy floral parts as available alternate food resources by the thrips appear interesting. The larvae inside the keel petals feeding on the nectar and pollen grains also accidentally resulted in the self-pollination of the flowers was observed in the case of *Arachis hypogea* (Hammons & Leuk 1966, Billes 1941). Dispersion of *M. distalis* in *Dolichos lablab* was influenced by the age of the flowers and also its quest for a mate and subsequent egg-laying by the females. These eggs laid in early immature buds provided adequate time for hatching. A similar observation was made by Hagerup (1950) where winged females migrated from flower to flower in search of apterous males resulting in the pollination of *Calluna*.

The papilionaceous flowers by their type of flower structure, form a suitable micro-environment for foraging, breeding and ovipositional site for thrips incidentally helping for an early pollination of these flowers. *Dolichos lablab* flowers although they are considered self-fertile, as a result of thrips flower association, both are mutually benefited, helping the flower in a early selfing as well as crossing between the flowers involving interfloral pollen movement leading to cross-fertilization. Thus an equilibrium status is maintained by the thrips between the inbreeding and outbreeding in the natural population of *Dolichos lablab*.

Acknowledgements

The authors are extremely thankful to Professor T N Ananthakrishnan, Principal Investigator of the Project for his valuable guidance and critical perusal of this manuscript. This work was done during the tenure of a Council of Scientific & Industrial Research Project.

References

Allard R W 1954 Natural hybridization in Lima beans in California; *Amer. Soc. Hort. Sci. Proc.* **64** 410–416

Ananthakrishnan T N 1982 Thrips and Pollination biology; *Curr. Sci.* **51** 168–172

——, Varatharajan R and Gopinathan K 1981 Seasonal periodicity of thrips infesting some compositae in relation to pollination; *Proc. Indian natn. Sci. Acad.* **B47** 811–815

Billes D J 1941 Pollination of *Theobroma cacao* L. in Trinidad. B W I; *Trop. Agric. Trin.* **18** 151–156

Fahn 1982 Nectaries: ultrastructure and secretion. Paper presented in the symposia held by Linnean Society of London in celebration of the centenary of Darwin's death

Gopinathan K and Varatharajan R 1982 On the morphology, Topography and the significance of stomata on floral nectaries of some compositae; *Phytomorphology* vol. 32 265–269

——, —— and Ananthakrishnan T N 1981 Incidence of *Microcephalothrips abdominalis* (Crawford) (Thysanoptera: Insecta) in relation to the pollination biology of the weed *Ageratum conyzoides* Linn. (Compositae); *Proc. Indian natn. Sci. Acad.* **B47** 505–509

Hegerup O 1950 Thrips pollination in *Calluna*; *D. Kgl. Danste Vidensk. Selsk. Biol. Medd.* **18** 1–116

Hammons R O and Leuck D B 1966 Natural cross pollination of the peanut *Arachis hypogaea* L. in the presence of bees and thrips; *Agron. Journ.* **58** 396

Lewis T 1973 *Thrips, their Biology, Ecology and Economic Importance* (London: Academic Press) 349 pp

Mackie W W and Smith F L 1935 Evidence of field hybridization in beans; *J. Am. Soc. Agron.* **27** 903–909

Varatharajan R, Gopinathan K and Ananthakrishnan T N 1982 Comparative efficiency of thrips in relation to other foraging insects in the pollination of *Cosmos bipinnatus* Cav. (Compositae); *Proc. Indian natn. Sci. Acad.* **B48** 735–739

Williams I H 1977 Behaviour of insects foraging on Pigeon pea (*Cajanus cajan* L (Mill sp.) in India; *Trop. Agric. (Trinidad)* **54** 353–363

Proc. Indian natn. Sci. Acad. B51 No. 5 pp 581–595 (1985)

Use of HCH (BHC) and Its Environmental Effects in India

K N MEHROTRA

Division of Entomology, Indian Agricultural Research Institute, New Delhi 110012

(Received 12 July 1985; after revision 18 October 1985)

HCH (hexachlorocyclohexane), popularly known as BHC, was introduced in India in 1949 to control insect pests of agricultural importance. Although, its main use is still in agriculture, it is also being used in the public health sector in smaller amounts since 1960. Ever since its introduction, 575 thousand tonnes of HCH have been used in the country till todate (nearly 500 thousand tonnes in agriculture and 75 thousand tonnes in public health sectors, respectively). The current annual consumption of HCH is nearly 36 thousand tonnes (30 thousand tonnes in agriculture and 6 thousand tonnes in public health). The large-scale usage of HCH has led to its near ubiquitous presence in the Indian environment, including human and animal tissues. The situation at present is not very alarming, but the continued use of HCH will certainly pose a problem in coming years. Although it has been claimed that the half-life of HCH in Indian environment is around three months as compared to 4–5 years in the temperate regions, there is a need to confirm these findings. Acute poisoning due to HCH has been reported from the various parts of the country more than once and there is a risk to health due to chronic exposure. There are indications that the presence of HCH residues in women can lead to abortions and premature delivery. Further, HCH is a known carcinogen. Keeping these facts in view there is an urgent need to phase out HCH from the Indian scene by 2000 AD. Besides, the residues of HCH should be monitored in various components of the environment in various agro-climatic zones of the country.

Key Words: HCH, Environmental pollution

India being a tropical country has more than its share of pests and diseases in both agriculture and public health (Mehrotra 1979a and b, 1983a). The importance of pesticides in controlling these pests is very well recognized even in integrated pest management (Mehrotra 1985). HCH, being cheap, reliable and broad spectrum insecticide was one of the first to be introduced in the country for use in agriculture. It was introduced in India in 1949 (Banerjee 1979). Although, HCH was introduced mainly for agricultural use, its use in public health sector, specially in the control of insect vectors of malaria, was started in 1960. The use in public health was mainly for controlling insect

vectors which had developed resistance to DDT. From the time of its introduction till todate nearly 575 thousand tonnes of HCH has been used in the country (500 thousand tonnes in agriculture and nearly 75 thousand tonnes in public health sector). Current annual consumption of HCH is around 36 thousand tonnes (30 thousand tonnes in agriculture and 6 thousand tonnes in public health sector). The projected demand of HCH is around 60 thousand tonnes annually by the year 1990 (39 thousand tonnes in agriculture and 21 thousand tonnes in public health sectors) (GOI 1984). As it is, HCH constitutes nearly 50% of the total pesticides used in the country and that too mostly in agricultural sector.

The use of HCH in agriculture on large scale has been mainly because of its certain peculiar properties: it is persistent with fumigant action, non-phytotoxic (except to cucurbitaceae) at the concentration normally used, but at higher concentrations may cause root deformation and polyploidy. It is generally more toxic than DDT to most insect pest species and can also be used as a soil insecticide. There are 5 important isomers of the compound, of which *gamma*-isomer or lindane has insecticidal properties whereas *beta*-isomer is the most stable. Commercially available HCH contains 13% of lindane. Keeping in view these properties it has been recommended for wide variety of crops and that is why the consumption of HCH has been going up since the time of its introduction in the country (table 1). There has been nearly 20-fold increase in HCH consumption from 1955 to 1980, a quantum jump in its consumption in agricultural sector was seen between 1965 and 1970 (table 1) as a result of the introduction of, and increase in area under, high-yielding varieties during this period.

HCH has been recommended in agriculture for controlling wide varieties of pests of paddy, maize, sorghum, cotton, sugarcane, groundnut, mustard and rapeseed, gram, pea and various plantation crops (ICAR 1961, 1981, GOI 1970). Usually the recommended doses of application of HCH are 3–5 kg a.i./hectare, but recently as high as 12.5 kg a.i./hectare has also been recommended (ICAR 1985 a and b). Because HCH is stable, highly fat-soluble and having low water-solubility, it tends to stay in the environment. In this respect it can be termed as an ideal environmental pollutant. Although the knowledge regarding the extent of environmental pollution due to pesticides including HCH in India is fragmentary, nevertheless, the subject has been reviewed from time to time by various workers in a number of symposia (Bindra & Kalra 1973, Agnihothrudu & Mithyantha 1978, Edwards et al. 1978, Raychaudhuri & Gupta 1979, Attri et al. 1982, Krishna Murti 1984). Scientific

Table 1 *Consumption of HCH (BHC) in India (in tonnes)*

Year	NMEP*	Agriculture	Total
1955	—	1603	1603
1960	644	3247	3891
1965	618	6873	7441
1970	750	16166	16196
1971	2250	14000	16250
1972	1500	15850	17350
1973	2250	18000	20250
1974	6000	18000	24000
1975	2689	21235	23924
1976	6400	18190	24590
1977	3780	24820	28600
1978	11000	24254	35254
1979	6853	24976	31829
1980	2150	26610	28760
1981	4588	23778	28366
1982	8717	23661	32378
1983	10632	21757	32389
1984	6519	29481	36000

*NMEP—National Malaria Eradication Programme

data collected so far to assess the environmental load of HCH in ecosystem relevant to India are summarized in tables 2–14.

HCH Residues in Soil

Soil has been recognised as the main sink of pesticides used in agriculture. In case of HCH it becomes still more important because it has been recommended as a soil insecticide for various crops (ICAR 1981). Despite the fact that it has been extensively used in agriculture, detailed information on the levels of residues of HCH in Indian soils is meagre. Venkataramaiah and Singh (1973) extensively studied the HCH residues in soils of the coffee plantations in Kallar region of Karnataka. Their study showed that the HCH residues ranged from 0.025 to 124 ppm up to 15 cm top soil and were not detectable below 60 cm. A survey of Punjab soils conducted in 1976-77 for pesticide residues showed that only one sample out of 91 had a detectable amount of HCH. However, a later study revealed extensive amounts of HCH levels in the soil samples collected from villages around Ludhiana (Kalra & Chawla 1983). Residues of HCH in Punjab soils ranged from traces to 0.053 ppm and that *alpha*, *beta*, *gamma* and *delta* isomers were present. Highest concentrations were those of *beta*-isomer. The persistence of HCH and its isomers in the soil under various crops has also been reported. Agnihotri et al. (1974) showed that γ HCH applied to the first 15 cm of the soil was lost within 6 months of its application. This, however, is in contrast to what Chawla and Chopra (1967) reported (i.e. 50% HCH remained in soil even after a 9-month period). A very fast dissipation of HCH from the clay loam soil has been reported (Srivastava & Yadav 1977). It has also been shown that HCH can be adsorbed by the soil particles and that adsorption is highest in acidic laterite soil followed by black, red and *kari* soils. Clay soils retain very little, if any, HCH. The adsorption of HCH on soil is dose-dependent. HCH is degraded in the soil, the rate of degradation being dependent on the moisture and organic contents of the soil (Wahid & Sethunathan 1980). It is degraded very fast under paddy soil conditions. However, *gamma* isomer of HCH is degraded faster than the *beta* isomer (Siddaramappa & Sethunathan 1975, 1976, Mathur & Saha 1977, Sahi et al. 1976, Pal et al. 1980). The half-life of HCH in Indian soils have been shown to be around 4 months (Agnihotri et al. 1977). This finding needs confirmation because had it been so, one should not encounter HCH residues in the environment.

HCH in Water

Despite the importance of pesticide residues in aquatic systems, very few studies have been made regarding the HCH residues in water Visweshwariah et al. (1976) analysed water samples collected from ponds in the coffee plantations at Chikmaglur district of Karnataka and showed the presence of HCH. The concentration of HCH ranged between 0.02 and 0.2 ppm, the insecticide, however, could not be detected in the silt of ponds of the area. Drinking water was shown to contain 0.02 – 6.16 ppm of HCH in the same area (Venkataramiah & Singh 1973). Kalra and Chawla (1981a) analyzed about 20 samples of water collected from Ludhiana and Muktsar in Punjab for pesticide residues. The mean HCH level detected was 0.9 ppb. The tap water, however, did not show any HCH.

HCH Residues in Cereals and Animal Feed

Cereals and pulses: Since the average Indian diet is predominantly vegetarian and the cereals along with pulses constitute its major portion, these commodities have been surveyed from time to time. Earlier survey by

Table 2 *Contamination of cereals and pulses with HCH residues in India*

Location Period	Samples analysed	Samples contaminated	Mean range (ppm)	Reference
		Wheat Grain		
Ludhiana (1979)	23	23	Tr.–4.0	Kalra & Chawla (1981a, 1983)
Ferozepur (1979)	9	9	0.02–32.4	,,
Sangrur (1979)	10	10	0.41–30.0	,,
Sangrur (1980)	26	26	0.2–10.50	,,
Bombay: Urban (1978)	21	6	5.1–13.9	Noronha et al. (1980)
Bombay: Rural (1979-80)	20	12	0.05–7.3	,,
		Wheat Flour		
Punjab (7 cities) (1976)	140	116	Tr.–12.0	Joia et al. (1978)
Sangrur & Ludhiana (1980)	24	22	Tr.–17.6	Kalra & Chawla (1983)
		Maize Grain & Flour		
Punjab	10	10	Tr.–1.0	Kalra & Chawla (1981a, 1983)
		Cereal & Cereal Products		
Hyderabad	594	144	Tr.–20.8	Lakshminarayana & Menon (1975) and Lakshminarayana (1980)
		Paddy & Rice		
Bombay: Rural (1979-80) Paddy	10	4	0.01–0.2	Noronha et al. (1980)
Bombay: Urban (1978) Rice	20	13	4.2–11.2	,,
Ludhiana (1980) Rice	10	10	Tr.–0.10	Kalra & Chawla (1981a, 1983)
		Sorghum & Bajra		
Bombay: Rural (1979-80) Sorghum	29	18	0.01–0.8	Noronha et al. (1980)
Bombay: Rural (1979–80) Sorghum	2	2	0.01–0.2	,,
		Pulses		
Hyderabad (1968-79)	161	16	Tr.–10.0	Lakshminarayana (1980)
Hapur	81	31	7–87	ICAR (1967)
Mysore	37		23.5	Majumdar (1973)
Ludhiana	10	8	Tr.–0.05	

Bindra et al. (1973) showed that 4 out of 54 samples of wheat grain had detectable amounts of HCH. Later work not only confirmed the existence of HCH residues in grains but also showed that it is prevalent in various parts of the country and that its concentrations were higher than those reported for DDT (Mehrotra 1985). The existence of HCH in Punjab on wheat grain, wheat flour, maize grain and flour has been reported by Kalra and Chawla (1981a, 1933). The survey of cereal and cereal products in Andhra Pradesh revealed that HCH was present in nearly 25% of the samples (Lakshminarayana & Menon 1975, Lakshminarayana 1980). Noronha et al. (1980) showed the presence of HCH residues in paddy, rice, sorghum and bajra in Maharashtra. The data presented in table 2 suggest that HCH is present in nearly every commodity and that its concentrations have gone up over the years. This may perhaps be due to increased use of HCH in agricultural sector. Considerable amounts of HCH residues in the spices exported from India have also been reported by Sullivan (1980) (table 3).

Vegetables and fruits: Extensive surveys for the pesticidal residues on vegetables and fruits have been conducted over the years in various regions of the country. The surveys have been conducted at Hyderabad (Lakshminarayana 1980), Punjab (Kalra et al. 1978, Kalra & Chawla 1983, Gupta & Kalra 1983), Karnataka (Visweshwaraiah & Jayaram 1972) and Haryana (Verma 1980). The data of these surveys are summarised in table 4. All the samples of leafy vegetables collected in Karnataka showed the presence of high levels of HCH. Okra, brinjal, cabbage, cauliflower, radish, tomato and turnip had HCH residues in more than 40% of the samples collected in Haryana and Andhra Pradesh. Similar results have also been reported from Punjab (Kalra & Chawla 1981a, 1983).

Table 3 *Residues of HCH in spices exported from India*

Commodity	HCH Residues (ppm)
Black pepper	0.2–0.22
Celery seed	0.8–0.68
Dill seed	0.14
Turmeric	0.04–0.6
Ginger	0.02–1.19
Fennel	0.01–3.52

After Sullivan (1980)

Vegetable oils and oilseeds: HCH residues were common in the market samples of vegetable oils of groundnut, mustard, sesame, coconut, cotton seed, rice bran, linseed and rapeseed collected from Punjab, Uttar Pradesh and Andhra Pradesh. The overall level of HCH residues varied from traces in Hyderabad to as high as 0.71 ppm in mustard oil in Lucknow (Siddiqui et al. 1980). Mustard oil was also found to be contaminated in Punjab where its level was 0.31 ppm (table 5). By and large, HCH residues in oils and oil seeds were of the same order as DDT (Mehrotra 1985). Despite the widespread occurrence of HCH in vegetable oils, hydrogenated oils were free of pesticide residues except at Lucknow where the HCH residues as high as 0.98 ppm were encountered (Siddiqui et al. 1980).

Milk and milk products: The data on residues of HCH in bovine milk and its products show a widespread occurrence of HCH in various parts of the country (tables 6 & 7). The residues of HCH were found to be highest in Hyderabad and in the rural areas of Ferozepur and Sangrur in Punjab. The milk was least contaminated in the urban areas of Punjab and Uttar Pradesh. An extensive survey of HCH residues in butter in various parts of the country undertaken by Kalra and Chawla (1981a, 1983) indicated that

Table 4 *HCH Residues in vegetables and fruits*

Locality Period	Samples analysed	Samples contaminated	Commodity	Level of Residues (ppm)	Reference
Ludhiana (1975-1976)	40	40	Potato	Tr.–0.05	Kalra et al. (1978)
(1979)	51	30	Potato Carrot raddish cauliflower okra tomato brinjal	Tr.–0.09	Gupta & Kalra (1983)
Karnataka	300	300	Leafy vegetables	10.5–20.0	Visweshwaraiah & Jayaram (1972)
Hyderabad (1968-79)	78	12	Leafy vegetables	Tr.–8.0	Lakshminarayana (1980)
	323	57	Starchy vegetables	Tr.–6.0	"
	847	55	Other vegetables	Tr.–4.0	"
	60	28	Grapes	Tr.–1.5	"
Hissar (1979)	66	12	Summer vegetables okra cucurbits brinjal	0.84–6.03	Verma (1980)
	68	9	Winter vegetables radish mustard tomato turnip	1.31–3.80	
	61	12	Mixed vegetables	Tr.–3.5	"

the butter was invariably contaminated with HCH in Punjab, Haryana, Uttar Pradesh, Rajasthan, Gujarat, Andhra Pradesh, West Bengal and Tamilnadu. Highest concentrations of HCH residues in butter were observed in West Bengal and Andhra Pradesh (table 7). Residues of HCH in butter were mainly due to β-HCH although *alpha* and *gamma*-isomers were also detected. By and large, the HCH residues in butter exceeded the maximum levels of HCH reported in 12 countries in the West. *Desi ghee* (clarified butter) invariably contained HCH residues (table 8). An interesting study by Kapoor et al. (1980) revealed that contamination of bovine milk with HCH was

Table 5 *Residues of HCH in the market samples of oils and oil seeds in India*

Commodity	Locality	Residue level (ppm)	Reference
		Oils	
Groundnut	Hyderabad	Tr.	Lakshminarayan (1980)
,,	Punjab	0.141	Battu et al. (1980)
,,	Lucknow	0.112–6.421	Srivastava et al. (1983)
,,	Sitapur	0.3–2.4	,,
Mustard	Punjab	0.310	Battu et al. (1980)
Sesame	,,	0.165	,,
Coconut	,,	0.02	,,
Cotton seed	,,	0.147	,,
Rice bran	,,	0.637	Kalra & Chawla (1983)
Linseed oil	,,	Tr.	,,
Rape seed oil	,,	Tr.	,,
Mustard oil	Lucknow	0.71	Siddiqui et al. (1980)
Hydrogenated oil	,,	0.98	,,
Coconut oil	,,	0.46	,,
		Seed	
Cotton seed	Punjab	0.79	Kalra & Chawla (1983)

Table 6 *Residues of HCH in bovine milk in India*

Locality	Samples analysed	Samples contaminated	Mean (ppm)	Reference
Ludhiana	14	14	0.05	Kalra & Chawla (1983)
Jullundur	10	10	0.03	,,
Ferozepur	16	16	0.40	,,
Sangrur	14	14	0.38	,,
Hyderabad			Traces—5.0	Lakshminarayana (1980)
Lucknow			0.057	Saxena & Siddiqui (1982)

Table 7 *Residues of HCH in butter in India*

Locality	Range
Punjab	
Amritsar	1.47–1.65
Bhatinda	0.76–0.83
Ludhiana	0.61–1.2
Sangrur	1.86–11.79
Gujarat	
Anand	1.33–1.59
Mehsana	1.51–2.48
Palanpur	0.62–1.32
Andhra Pradesh	
Hyderabad	0.8–8.2
Uttar Praddsh	
Aligarh	3.5–3.8
Bareilly	0.35–0.46
Tamil Nadu	
Coimbatore	0.98–1.29
Madras	1.33–1.42
Others	
Calcutta (W.B.)	3.14–5.69
Ratlam (M.P.)	0.71–1.2
Bharatpur (Rajasthan)	1.24–2.13
Rohtak (Haryana)	1.74–1.95
Bangalore (Karnataka)	1.18–2.1
Chandigarh	1.28–1.63
Delhi	0.61–0.9

According to Kalra and Chawla (1981a, 1983)

Table 8 *Residues of HCH in ghee samples from various parts of the country*

Location	Residues (ppm)
Andhra Pradesh (1978)	6.4
Punjab	2.2–6.8
Rajasthan	2.8
Uttar Pradesh	3.5–5.8

Source: Sandhu (1979), Kalra et al. (1983) and Lata et al. (1984)

Table 10 *Tissue distribution of HCH in goat, buffalo and chicken*

Tissue	Mean residue level (ppm)		
	Chicken	Goat	Buffalo
Muscle	0.109	0.018	0.012
Brain	0.162	0.068	0.076
Liver	0.195	0.032	0.038
Body fat	3.879	0.536	0.165
Bone marrow		0.203	0.252

Source: Kaphalia and Seth (1981)

Table 9 *HCH residues in the market samples of meat, poultry, fish and eggs*

Commodity	Locality period	Range (ppm)	References
Meat	Hyderabad (1967–79)	Tr.–2.0	Lakshminarayana (1980)
Chicken	Lucknow	0.014–14.104	Kaphalia & Seth (1981)
Chicken	Ludhiana	0.001–0.06	Kalra & Chawla (1983)
Goat	Lucknow	0.01–1.522	Kaphalia & Seth (1981)
Goat	Ludhiana	0.004–0.496	Kalra & Chawla (1983)
Sheep	Ludhiana	Tr.–0.21	"
Buffalo	Lucknow	0.006–0.485	Kaphalia & Seth (1981)
Pig	Ludhiana	Tr.–0.21	Kalra & Chawla (1983)
Fish	Ludhiana	Tr.–1.12	"
Eggs	Bombay	0.14–1.01	Banerji (1979)
Eggs	Ludhiana	0.06–0.62	Kalra & Chawla (1983)

partly due to the use of HCH in malaria control programme.

Meat and poultry: Samples of meat of goat, sheep, buffalo, pig, chicken and fish show the presence of significant amounts of HCH residues (tables 9 & 10). The residues were comparatively higher in chicken meat in Lucknow than in Ludhiana. Similarly, goat meat showed more HCH residues in Lucknow than in Ludhiana. A detailed study by Kaphalia and Seth (1981) reported the presence of high amounts of HCH in various body tissues of goat, buffalo and chicken collected in and around Lucknow. Chicken, by and large had highest concentration of HCH residues when compared to goat and buffalo. Of the various tissues highest concentration of HCH was seen in body fat of chicken followed by goat and least in buffalo. Eggs in Andhra Pradesh and Punjab were found to contain HCH residues (Lakshminarayana 1980, Kalra et al. 1978). Recently in Punjab 10 out of 13 samples of eggs analyzed for residues of HCH showed its presence at concentrations ranging from 0.06 to 0.62ppm (Kalra & Chawla 1983).

The residues of HCH in different fishes of Punjab has been reported by Kalra and Chawla (1983). Mean residue levels (ppm) of HCH detected were 0.031 in *Singhara*, 0.123 in *Malhi*, 0.247 in *Rohu*, 0.059 in *Sohal*, 0.033 in *Khangra*, 0.026 in *Sarmahi* and 0.149 in *Chakar*. Bulk of these residues consisted of *alpha*, *beta* and *gamma*-isomers of HCH.

Animal feed: Because of the importance of residues in animal feed in contaminating animal products including milk it is important to know the HCH residues in animal feed. It is rather unfortunate that very limited information is available on this point in the various parts of the country. Samples of oil cake which is most commonly used as animal feed, when analysed, revealed the presence of HCH. It was present in cotton seed cake (0.05 ppm) in Ludhiana, (0.27ppm) Muktsar and (0.068 ppm) Ferozepur. Mustard seed cake was contaminated at a level of 0.068 ppm in Ludhiana and groundnut seed cake to the extent of 0.03 ppm. The residues consisted mainly of α-isomer and the other isomers, β and γ were present in very small quantity (Kalra & Chawla 1981a). The residues of HCH in other animal feeds like wheat straw and fodder were also observed (Kaphalia 1982). The highest level of HCH in wheat straw was found to be 6.6 ppm and in wheat bran 1.69 ppm. The residues in other products are given in table 11.

HCH Residues in Wild Life

The residues of HCH have been reported from the wild birds namely, egret, crow, kite and vulture. The residues were detected in nearly every tissue of the body. Blood plasma and brain usually had higher concentration of HCH when compared to DDT. Although all the three isomers—*alpha*, *beta* and *gamma* were detected, the *gamma*-isomer predominated (Kaphalia et al. 1981).

Table 11 *HCH residues in animal feed sample in Punjab*

Commodity	No. of samples analysed	No. of samples contaminated	Mean residue level (ppm)	Range (ppm)
Gram seed husk	16	16	0.172	0.02–0.837
Wheat straw	32	32	0.67	Tr.–6.6
Wheat bran	4	4	1.69	Tr.–2.9
Mixed straw and Fodder	4	4	0.20	0.06–0.71
Fodder	4	4	0.10	0.01–0.29

Source: Kalra and Chawla (1983)

Table 12 *HCH residues in the human tissues in India*

Location and year	No. of samples analysed	No. of samples contaminated	Level of residue (ppm)		Reference
			Mean	Range	
			Adipose tissue		
Bangalore	67	—	7.01	0.26–94.54	Chatterjee (1979)
			5.05		Gupta et al. (1982)
Bombay	34	—	1.62	0.2–7.23	Chatterjee (1979)
Calcutta	45	45	1.6	0.13–4.84	Gupta et al. (1980)
			0.45		Mukherjee et al. (1980)
Chandigarh	10	—	2.4	0.22–11.03	Chatterjee (1979)
Lucknow	—	—	2.3		Kaphalia & Seth (1979, 1983)
Ludhiana	51	51	4.3	Tr.–30.05	Chawla et al. (1980)
			Blood		
Lucknow					
Children			0.038		Kaphalia & Seth (1979, 1983)
Female			0.034		Siddiqui et al. (1981 a & b)
Male			0.075		

Table 13 *HCH residues in the milk of lactating females*

Locality	Level of residue (ppm)		References
	Mean	Range	
Ludhiana	0.108	Tr.–0.82	Kalra & Chawla (1981b; 1983)
Muktsar	0.220	0.02–0.77	,,
Lucknow	0.14		Siddiqui et al. (1981b)
Lucknow	0.20		Saxena & Siddiqui (1982)

Table 14 *HCH and its isomer residues in whole cooked meal samples*

Diet samples	No. of samples	Residues in micrograms/meal			
		α HCH	γ HCH	β HCH	Total HCH
Vegetarian					
Mean	20	6.45	3.26	9.88	19.59
Range		(0.37–14.28)	(0.14–8.38)	(1.25–23.39)	(1.77–42.18)
Non-vegetarian					
Mean	20	7.28	2.86	13.12	23.26
Range		(0.80–12.36)	(0.16–6.08)	(2.02–29.13)	(2.98–47.57)

Source: Gupta et al. (1982b)

HCH Residues in Human Tissues

Near ubiquitous presence of HCH residues in the environment and its property of bio-amplification through food chain leads to the accumulation of significant amounts of HCH residues in the human tissues. The residues of HCH have been reported from adipose tissue (Dale et al. 1965, Chawla et al. 1978, Chatterjee 1979, Gupta et al. 1980, 1982a, Kaphalia & Seth 1979, 1983), blood (Kaphalia & Seth 1979, Kaphalia 1983), cerebro-spinal fluid (Kaphalia & Seth 1979) and in the umbilical cord blood collected between 24 to 48 hr after child birth (Siddiqui et al. 1981a). The information on the occurrence of HCH residues in human tissues is summarized in table 12. It is evident that there are tremendous variations in the HCH residues in human adipose tissues in various parts of the country. The limited studies conducted so far suggest that higher concentrations of HCH residues are seen in Bangalore than in Bombay, Calcutta, Chandigarh or Lucknow. In Punjab the HCH residues were seen in all the fifty samples of adipose tissues analysed. There were more HCH residues in the samples obtained from rural populations than urban populations. The HCH residues mainly consisted of *alpha*, *beta* and *gamma*-isomers and the *beta*-isomer usually predominated. Sex or dietary habits did not significantly influence the concentration of HCH residues. It has been suggested that wherever β-isomer is predominant it is due to the old exposure to HCH. In newly exposed individuals α and γ-isomers of HCH predominate (Kalra & Chawla 1983).

Besides the adipose tissues the presence of large amounts of HCH in the milk of the lactating women have been reported from various parts of the country (Kalra & Chawla 1981b, 1983, Siddiqui et al. 1981 a & b, Saxena & Siddiqui 1982) (table 13). The presence of such high concentrations of HCH in the breast milk collected from lactating mothers after child birth is really alarming as the neonates consuming such a breast milk will have a significantly high intake of HCH (Siddiqui et al. 1981b).

The high concentrations of HCH seen in human tissues are because of the presence of high amounts of residues in the diet. It has been estimated that the daily intake of HCH in an average Indian vegetarian diet is 19.59 μg and that of non-vegetarian diet is 23.26 μg (table 14). This amount of HCH in the Indian diet is 2 to 20 times higher than the corresponding diets in other parts of the world (table 15).

Hazards due to HCH Residues in the Environment

The concentrations of HCH present in the environment, although not actually toxic,

Table 15 *Average dietary intake of HCH residues in various countries*

(μgm/person/day)

Country	HCH isomer	Gamma HCH
Australia	—	Tr.
Canada	2.5	2.0
Czechoslovakia	53.0	—
East Germany	—	10
Italy	16.7	5.4
U.K.	—	4.4
U.S.A.	1.1	0.2
Yugoslovakia	62.0	—
India:		
Vegetarian	57.0 (135)*	10.0 (27)*
Non-Vegetarian	72.0 (147)*	9.0 (20)*

*Figures in parenthesis are the estimates of the maximum values of dietary intake of HCH

certainly pose a problem of chronic toxicity. The mammalian toxicity of the various isomers of HCH differs tremendously; *alpha* HCH has low acute and chronic toxicity but has cumulative toxicity (acute oral LD_{50} for rat being 500 mg/kg body weight); *beta*-HCH has low acute but has high chronic and cumulative toxicity (acute oral LD_{50} for rat being 6000 mg/kg body weight); *gamma*- HCH has low acute and chronic toxicity (acute oral LD_{50} being 125 to 200 mg/kg body weight); *delta*-HCH has low acute and chronic toxicity but it is irritant to mucous membranes (acute oral LD_{50} for rat being 900 mg/kg body weight). Keeping this in view the recommended ADI (acceptable daily intake) is 0.005 mg/kg body weight for *alpha*-HCH, 0.001 mg/kg body weight for *beta*-HCH and 0.0125 mg/kg body weight for *gamma*-HCH (Martin 1957, Negherbon 1959). The ICAR (1967) had recognised some of the incidences of HCH poisoning to fish, wild life and plant protection workers. Acute poisoning due to HCH has been recorded in India on large scale a number of times (Gupta 1975, Khare et al. 1977, Nag et al. 1977, Misra & Nag 1984). Acute poisoning due to HCH leads to epileptic, grand mal, petit mal, myoclonic and mixed type of seizures and neurocular manifestations. HCH is also known to induce spontaneous abortions and abnormal delivery (Saxena et al. 1980, 1981). HCH is also a known carcinogenic chemical. From the safety point of view DDT is far safer than HCH.

Conclusions and Recommendations

(1) Till todate 500 thousand tonnes of HCH has been used in agricultural sector and nearly 75 thousand tonnes in public health sectors. This has led to development of resistance in insect vectors of malaria and a few insect pests of agricultural importance.

(2) HCH is present in various components of the environment. The extent of HCH residues are at present not very high but are showing increasing trend in certain parts of the country specially in milk and milk products. This should be a cause of concern and ways and means may be found to decrease the environmental load of HCH.

(3) Significant amounts of HCH are seen in the human and wild life tissues. This should be a cause of concern.

(4) Repeated demonstration of the occurrence of high amounts of HCH in the milk of lactating women is a matter of grave concern and should be immediately looked into.

(5) HCH constitutes nearly 50% of the total pesticides consumed in the country. There is an urgent need for scientific studies to find out suitable substitutes to replace this insecticide in the agricultural sector. It should be phased out in such a manner so that it is totally eliminated from the environment in the next 5 to 10 years and certainly by 2000 AD.

(6) It is urged that HCH and other pesticides be monitored in various agro-climatic zones and urban areas of the country on a permanent basis.

References

Agnihotri N P, Pandey S Y and Jain H K 1974 Persistence of BHC and aldrin in soil and translocation in *moong* and *lobia*; *Indian J. Ent.* **36** 261–268

——, ——, —— and Srivastava P K 1977 Persistence of aldrin, dieldrin, lindane, heptachlor and DDT in soil; *J. Ent. Res.* **1** 89–91

Agnihothrudu V and Mithyantha M S 1978 *Pesticide Residues—A Review of Indian Work* (Bombay: Rallis India Limited) 173 pp

Attri B S, Ramanathan N L and Krishna Murthi C R 1982 *Biodegradable Pesticides* (New Delhi: Government of India) 350 pp

Banerji S A 1979 Organochlorine pesticides residues; *Scavenger* **9** 2–7

Banerjee S N 1979 Agricultural production through increased pesticide usage; *Pesticide Information* **4** (4) 17–19

Battu R S, Chawla R P and Kalra R L 1980 Insecticide residues in market samples of vegetable oils and oilseeds cakes from selected areas of the Punjab; *Indian J. Ecol.* **7** 1–8

Bindra O S and Kalra R L 1973 A review of the work done in India on pesticide residues; in *Progress and Problems in Pesticide Residue Analysis* pp 1–44 eds. O S Bindra and R L Kalra (Ludhiana: Punjab Agricultural University)

——, Udeaan A S and Deshmukh S N 1973 Pesticidal contamination of wheat grains in Pubjab; in *Progress and Problems in Pesticide Residue Analysis* pp 56–60 eds. O S Bindra and R L Kalra (Ludhiana: Punjab Agricultural University)

Chatterjee S K 1979 Report on the project entitled *Study of Pesticide Pollution from Chlorinated Pesticides specially DDT in the Environment of Man and Livestock in the Country* (New Delhi: Department of Science and Technology)

Chawla R P and Chopra S L 1967 Persistence of residues of DDT and BHC in normal and alkali soils; *J. Res. Punjab Agric. Univ.* **4** 96–103

——, Sharda B M, Joia B S and Kalra R L 1978 A survey of residues of organochlorine insecticides and biopsy human fat samples; in *Pesticides Residues in the Environment in India* pp 62–69 eds. C A Edwards, G K Veeresh and H R Kreuger (Bangalore: University of Agricultural Sciences)

Dale W E, Copeland M F and Hayes W J 1965 Chlorinated pesticies in body fat of people in India; *Bull. Wld. Hlth. Org.* **3** 471–477

Edwards C A ,Veeresh G K and Kreuger H R 1978 *Pesticides Residues in the Environment in India*; (Bangalore: University of Agricultural Sciences Tech. Series No. 32) 519 pp

GOI 1970 *Plant Prot. Bulletin* **22** 1–101 (New Delhi : Government of India)

—— 1984 Report of the working group on pesticide industry (New Delhi: Ministry of Chemicals and Fertilizers, Government of India)

Gupta P C 1975 Neurotoxicity of chlorinated hydrocarbon insecticide poisoning. A clinical and electroencephalographic study of man; *Indian J. Med. Res.* **63** 601–606

Gupta S C and Kalra R L 1983 Insecticide residues in market farm-gate samples of vegetables from Ludhiana, Punjab; *Indian J. Ecol.* **10** 20–24

Gupta S K, Patel J S, Shah M P, Jani J P, Chatterjee S K and Kashyap S K 1980 Organochlorine insecticide residues in fat of people from urban centres in India; *Pesticide* **14** 3-4

——, Srivastava N, Patel J S, Shah M P, Jani J P, Chatterjee S K and Kashyap S K 1982a Organochlorine insecticide residues in the fat of people from urban centres in India, II; *Pesticides* **16** 11–13

——, Vergese S, Chatterjee S K and Kashyap S K 1982b Organochlorine insecticide residues in cooked meal samples in India; *Pesticides* **16** 8–9

ICAR 1961 *Handbook of Agriculture* (New Delhi: Indian Council of Agricultural Research) pp 502–562

—— 1967 Report of the Special *Committee on Harmful Effects of Pesticides* (New Delhi: Indian Council of Agricultural Research) 93 pp

—— 1981 *Handbook of Agriculture* pp 417–503 (New Delhi: Indian Council of Agricultural Research)

—— 1985a *Research Highlights* (New Delhi: Indian Council of Agricultural Research)

—— 1985b *Annual Report* 1984-85 (New Delhi: Indian Council of Agricultural Research)

Joia B S, Chawla R P and Kalra R L 1978 Residues of DDT and BHC in wheat flour in Punjab; *Indian J. Ecol.* **5** 120–127

Kalra R L and Chawla R P 1981a Monitoring of pesticide residues in the Indian environment; in *Indian Pesticide Industry — Facts and Figures* ed B V David (Bombay: Vishvas Publications)

—— and —— 1981b Occurrence of DDT and BHC residues in human milk in India; *Experientia* **37** 404–405

—— and —— 1983 Studies on pesticide residues and monitoring of pesticide pollution — Final Technical Report (Ludhiana: Punjab Agricultural University) 230 pp

——, ——, Dhaliwal G S and Joia B S 1978 DDT and HCH residues in foodstuffs in Punjab; *Proc. Symp. Nuclear Technique in Studies of Metabolism, Effects and Degradation of Pesticides* pp 9–30 (Bombay: Department of Atomic Energy)

——, ——, Sharma M L, Battu R S and Gupta S C 1983 Residues of DDT and HCH in butter and ghee in India 1978–1981; *Environ. Pollut.* (Series B) **6** 195–206

Kaphalia B S 1982 Organochlorine pesticide contamination in some species of fodder grass; *Environ. Pollut.* (Series B) **3** 231–237

—— 1983 Chlorinated pesticide residues in blood plasma and adipose tissues of normal and exposed human populations; *Indian J. Med. Res.* **71** 245–246

—— and Seth T D 1979 Isomers and metabolites of DDT and BHC in blood plasma, adipose tissue and cerebrospinal fluid (CSF) in persons of Lucknow; *Indian J. Biochem. Physiol.* **15** 79

—— and —— 1981 DDT and BHC residues in some body tissues of goats, buffaloes and chicken at Lucknow, India; *Pestic. Monit. J.* **15** 103–106

——, Hussain M M, Seth T D, Kumar A, Krishna Murthi C R 1981 Organochlorine pesticide residues in some Indian wild birds; *Pestic. Monit. J.* **15** 9–13

Kapoor S K, Chawla R P and Kalra R L 1980 Contamination of bovine milk with DDT and HCH residues in relation to their usage in malaria control programme; *J. Env. Sci. Hlth.* **B15** 545–557

Khare S B, Rizvi A G, Shukla O P, Singh R R P, Prakash O, Misra V D, Gupta J P and Sethi P K 1977 Epidemic outbreak of neurocular manifestations due to chronic BHC poisoning; *J. Assoc. Phys. India* **25** 215–222

Krishna Murti C R 1984 *Pesticide Residues in Food and Biological Tissues* pp 1–76 (New Delhi: Indian National Science Academy)

Lakshminarayana V 1980 Screening for organochlorine insecticide residues in samples of foodstuffs drawn from Hyderabad-Secunderabad markets. Paper presented at the meeting of the ICAR Committee to review the pesticide residues problems in the country, held at Ludhiana on April 14-15, 1980

—— and Menon P K 1975 Screening of Hyderabad market samples of food-stuffs for organochlorine insecticides residues; *Indian J. Pl. Prot.* **3** 14–19

Lata S, Siddiqui M K J and Seth T D 1984 Chlorinated pesticides in *desi ghee*; *J. Food Sci. Tech.* **21** 94

Majumdar S K 1973 Implications of pesticide residues in food and development of selective protectants; in *Progress and Problems in Pesticide Analysis* pp 10–32 eds. O S Bindra and R L Kalra (Ludhiana: Punjab Agricultural University)

Martin H 1957 *Guide to the Chemicals Used in Plant Protection* (London: Canada Department of Agriculture)

Mathur S P and Saha J G 1977 Degradation of lindane in a mineral soil and in the organic soil; *Bull. environ. Contam. Toxicol.* **17** 424–430

Mehrotra K N 1979a Rural prosperity through pesticides — future trends; *Pesticide Information* **4** 80–86

—— 1979b Plant Protection; in *50 years of Agricultural Research and Education* pp 59–64 (New Delhi: Indian Council of Agricultural Research)

—— 1983a Benefits and hazards of pesticides to society; *Pesticide Information* **9** 38–42

—— 1983b Major achievements through research in plant protection; in *Agricultural Research and Education Systems for Development* pp 359–362 eds. A P Saxena and V S Bhat (New Delhi: Indian Council of Agricultural Research)

—— 1985 Use of DDT and its environmental effects in India; *Proc. Indian natn. Sci. Acad.* **B51** 169–184

Misra U K and Nag D 1984 Accidental BHC poisoning in Uttar Pradesh, India; in *Facets of Environmental Problems* pp 47–50 ed C R Krishna Murti (New Delhi: Indian National Science Academy)

Mukherji D, Ghosh B N, Chakraborty J and Roy B R 1980 Pesticide residues in human tissues in Calcutta; *Indian J. Med. Res.* **72** 583–587

Nag D, Singh G C and Sanon S 1977 Epilepsy epidemic due to benzahexachlorine; *Trop. Geogr. Med.* **29** 229–232

Negharbon W O 1959 *Handbook of Toxicology Vol.* 3 (London: W B Saunders)

Noronha A B C, Khandekar S S and Banerji S A 1980 Survey of organochlorine pesticide residues in cereals obtained from Bombay markets and fields of its hinterland; *Indian J. Ecol.* **7** 165–170

Pal S S, Misra A K, Sethunathan M 1980 Inhibition of the reduction of flooded soils by hexachlorocyclohexane; *Soil Sci.* **129** 54–57

Raychaudhuri S P and Gupta D S 1979 *Environmental Pollution and Toxicology* (New Delhi: Today and Tomorrow's Publishers) 341 pp

Sahi B P, Rai M and Haque M M 1976 Effects of some soil applied insecticide on microbial populations in soils of sugarcane belt in North Bihar; *Indian Sugar* **26** 203–204

Sandhu T S 1979 Pesticide residues in food. Paper presented at the Indian Dairy Association's Seminar on Quality Control of Milk held at Delhi on November 9–10

Saxena M C and Siddiqui M K J 1982 Pesticide Pollution in India: organochlorine pesticides in women, buffalo and goat milk; *J. Dairy Sci.* **65** 430–434

——, ——, Bhargava A K, Seth T D, Krishna Murti C R and Kutty D 1980 Role of chlorinated hydrocarbon pesticides in abortions and premature labour; *Toxicology* **17** 323–331

——, Seth T D, Krishna Murti C R, Bhargava A K and Kutty D 1981 Organochlorine pesticides in specimens from women undergoing spontaneous abortion, premature full-term delivery; *J. Anal. Toxic.* **5** 6–9

Siddaramappa R and Sethunathan N 1976 Volatilization of lindane and water in soil free and flooded soil systems; *J. environ. Sci. Hlth.* **11** 119–217

—— and —— 1975 Persistence of gamma-BHC and beta-BHC in Indian rice soils under flooded conditions; *Pestic. Sci.* **6** 395–403

Siddiqui M K J, Seth T D and Saxena M C 1980 Organochlorine pesticides in edible oils; *Pesticide Annual* 108–110

——, Bhargava A K, Krishna Murti C R and Kutty D 1981a chlorinated hydrocarbon pesticide in blood of new born babies in India; *Pestic. Monit. J.* **15** 77–79

——, Seth T D, Krishna Murti C R and Kutty D 1981b Agrochemicals in maternal blood, milk and cord blood, a source of toxicants for prenates and neonates; *Environ. Res.* **24** 24–32

Srivastava B P and Yadav P R 1977 Dissipation of BHC in clay loam soil under the cover of maize crop; *Indian J. Pl. Prot.* **5** 62–69

Srivastava S, Siddiqui M K J and Seth T D 1983 Organochlorine pesticide residues in groundnut oil; *J. Food Sci. Technol.* **20** 25–27

Sullivan J H 1980 Pesticide residues in important spices. A survey of chlorinated hydrocarbons; *J. Agric. Food Chem.* **28** 1031–1034

Venkataramaiah G R and Singh D M B 1973 Pesticide residues in coffee plantations; *J. Plantation Crops* **1** 153–157

Verma A N 1980 Review of work done on pesticide residues in Haryana. Paper presented at the meeting of ICAR committee to review the pesticide residue problems in country, held at Ludhiana on April 14-15, 1980

Visweshwaraiah K and Jayaram M 1972 The effect of processing in lowering BHC residues in green leafy vegetables; *Pestic. Sci.* **3** 345–349

——, —— and Venkataramaiah G H 1976 Investigations on BHC residues in drinking water from coffee plantations and coffee beans; *J. Coffee Res.* **3** 96–101

Wahid P A and Sethunathan N 1980 Sorption-desorption of lindane by anaerobic and aerobic soils; *J. Agric. Food Chem.* **28** 623–625

Proc. Indian natn. Sci. Acad. B51 No. 5 pp 596-608 (1985)

Frequency of Stomata in Leaves of Young and Adult Palms

MONORANJAN GHOSE, B M JOHRI[1] and T A DAVIS[2]
Indian Statistical Institute, Calcutta 700035

(Received 5 August 1985)

A comparative study has been made with reference to frequency of stomata per unit area, their length on the abaxial and adaxial epidermis, width of individual epidermal cells, and stomatal index between young and adult plants of ten species of palms: *Areca triandra*, *Caryota urens*, *Chrysalidocarpus lutescens*, *Hyphaene dichotoma*, *Livistona rotundifolia*, *Phoenix pusilla*, *P. reclinata*, *Roystonea regia*, *Salacca zalacca* and *Veitchia merrillii*. The stomatal index and the number of stomata per unit area increase significantly in most of the adult plants. The index value decreases in adult *Phoenix pusilla*. The length of guard cells decreases significantly in two adult plants with isolateral leaves, but increases in others. The width of epidermal cells decreases significantly in most of the palms except the arecoid major group. Negative associations exist between the stomatal frequency and the size (length) of stomata (in two plants), and between the stomatal frequency and the width of epidermal cells (in six plants). There is hardly any resemblance between the sequence of the species according to Moore's (1973) systematic classification of palms, and the sequence according to the frequency of stomata.

Key Words: Palms, Stomata, Leaves, Epidermal cells, Stomatal index

Introduction

The study of stomata in palms has received much attention. Most of the work is confined to the leaves of adult palms (Tomlinson 1961, Bavappa 1966, Mahabale 1982, Mahabale & Shirke 1967, Trivedi & Upadhyay 1979). There is however, very little work on young palms (Ghose & Davis 1973, Basu & Basu 1978). The present article is an extension of a previous work (Ghose & Davis 1973) where it has been shown that significant differences in epidermal characters between young and adult palms exist. Therefore, the objective of the present article is (*a*) to confirm the earlier findings, and (*b*) to find out the range of variations between young and adult plants in respect to stomatal

[1]Department of Botany, University of Delhi, Delhi 110007
[2]J B S Haldane Research Centre, Nagercoil 4, Tamil Nadu

frequency, length of guard cells, stomatal index, and width of epidermal cells in ten different species of palms.

Materials and Methods

Epidermal peelings of lamina were prepared from seedlings, and adult forms of ten species of palms (table 1) according to the method described elsewhere (Ghose & Davis 1973). Most of the seedlings were raised from seeds collected from the Indian Botanic Garden, Howrah. The seeds of *Veitchia* were collected in Australia. Seeds were sown at different times according to their availability. The age of the seedlings ranged from 8 to 19 months. Some of the samples from adult specimens were collected from the Indian Botanic Garden, Howrah, and the garden of the Legislative Assembly, Calcutta, and the remaining from the premises of the Indian Statistical Institute.

In pinnate-leaved palms pieces of lamina were collected approximately half-way between the base and apex of a leaflet, inserted about half-way from the base to the apex of rachis. In palmate-leaved palms, or in seedling leaves which had not yet started splitting into leaflets, collections were in a region approximately half-way between the base and apex of a sector from one side of lamina. The species examined are tabulated according to Moore's (1973) classification of palms (table 1). Of the ten species studied, *Hyphaene dichotoma*, *Phoenix pusilla* and *P. reclinata* have isolateral leaves and the rest dorsiventral.

Results

The mature stomata of palms are of the tetracytic type. Each stoma is surrounded by four subsidiary cells, two terminal or polar cells, and two lateral cells. Terminal subsidiary cells are short and wide, while lateral ones are long and narrow and lie parallel to guard cells. Each guard cell has two cutinized ledges. Guard cells in *Caryota urens* are provided with transverse cuticular striations (figure 1A).

Stomatal Frequency

The total number of stomata in a field (obtained by using a lens-combination of 7×40) was counted. The area of each field was calculated as 0.17 sq. mm. Ten different fields per sample were examined at random. The data presented in table 1 are the mean for one field covering 0.17 sq. mm. For all the species, figures relating to young as well as adult palms are given.

Table 1 shows that the number of stomata, per unit area, increases in adult plants. *Livistona rotundifolia* registers over 339%, *Phoenix reclinata* over 179%, and *Veitchia merrillii* (figure 1B, C) over 109% increase in the number of stomata on abaxial surface. Stomata are absent on the adaxial epidermis of young as well as adult *V. merrillii* (figure 1D). Stomatal distribution on both the surfaces is almost similar in *H. dichotoma*. The stomata are totally absent on the adaxial epidermis of adult palms, but in young palms they are present in small numbers in *Areca triandra* and *L. rotundifolia* (figure 2A). The adaxial epidermis contains scattered stomata in *C. urens* (figure 2B), *Chrysalidocarpus lutescens* (figure 2C), *L. rotundifolia* (figure 2A), *Roystonea regia*, and *Salacca zalacca*. In young plants of *P. pusilla* and *P. reclinata* (figures 2D, 3A) the adaxial frequency is slightly less-than-half that of abaxial. In adult *P. reclinata* the frequency is almost the same on both surfaces.

Length of Guard Cells

The length of guard cells shows an increasing trend as the palms grow (table 2), the exceptions are *Hyphaene dichotoma* (figures 3B, C) and *Phoenix reclinata*. Among young plants the guard cells are the longest

Table 1 *Stomatal frequency in palm leaves (per unit area of 0.17 sq. mm)**

Major groups and species	Young plants		Adult Plants	
	Abaxial epidermis	Adaxial epidermis	Abaxial epidermis	Adaxial epidermis
I. Coryphoid				
Livistona rotundifolia (Lam.) Mart. (10)	27.7±0.47	3.1±0.57	121.8±1.60	Nil
II. Phoenicoid				
Phoenix pusilla Gaertn. (16)	31.9±1.77	13.2±0.87	39.3±0.19	14.7±0.89
P. reclinata Jacq. (8)	27.3±2.26	11.9±1.82	76.3±2.05	73.2±1.75
III. Borassoid				
Hyphaene dichotoma (white) Furtado (15)	14.2±0.94	11.1±0.72	27.2±0.81	28.5±0.58
IV. Lepidocaryoid				
Salacca zalacca (Gaertn.) Yoss (12)	20.6±0.62	Occasional —	30.2±0.96	0.8±0.36
VI. Caryotoid				
Caryota urens L. (12)	9.9±0.46	1.6±0.34	17.1±1.00	1.9±0.38
XII. Arecoid				
Areca triandra Roxb. (19)	10.5±0.89	0.2±0.13	15.4±0.94	Nil
Chrysalidocarpus lutescens H.A. Wendl. (16)	25.2±1.02	1.2±0.88	46.5±2.20	0.9±0.90
Roystonea regia (H.B.K.) Cook (11)	19.0±0.67	1.5±0.48	35.6±0.76	0.5±0.34
Veitchia merrillii (Becc.) Moore (10)	14.1±0.93	Nil	29.6±1.22	Nil

The classification shown above is that of Moore (1973). The age of the plant in months is given in parentheses after the binomial

*Average for 10 observations

Table 2 *Length of guard cells (μm) in palm lamina**

Species	Young plants		Adult plants	
	Abaxial epidermis	Adaxial epidermis	Abaxial epidermis	Adaxial epidermis
Livistona rotundifolia	16.7±2.13	18.0±0.46	19.7±0.53	Nil
Phoenix pusilla	23.4±3.16	25.1±0.41	27.2±0.59	27.2±0.47
P. reclinata	22.1±0.40	22.6±0.50	18.6±0.28	19.4±0.38
Hyphaene dichotoma	39.7±0.50	38.0±0.66	37.1±0.81	36.9±0.63
Salacca zalacca	28.0±0.38	26.0±0.84	33.9±0.48	33.7±0.72
Caryota urens	37.4±2.97	38.5±0.38	41.4±0.76	43.9±0.40
Areca triandra	33.5±0.38	33.5±0.52	36.5±0.69	Nil
Chrysalidocarpus lutescens	22.6±0.50	23.1±0.53	27.0±0.60	26.7±0.58
Roystonea regia	30.5±0.48	29.9±1.83	37.3±3.43	33.3±0.38
Veitchia merrillii	26.7±1.23	Nil	33.6±0.65	Nil

*Average for 10 observations

in *H. dichotoma* (figure 3B), and shortest in *Livistona rotundifolia* (figure 2A). In adult plants the length of guard cells is maximal in *Caryota urens* (figure 1A), and minimal in *P. reclinata*. The length of guard cells between abaxial and adaxial surfaces (when present) does not differ significantly, both in young and adult palms, except in adult *Roystonea regia*.

Stomatal Indices

The stomatal index was calculated from abaxial epidermis of both young and adult plants (table 3). Among the young plants, the lowest index value is in *L. rotundifolia*, and highest in *Salacca zalacca*. In adult plants the minimum value is in *Phoenix pusilla*, and maximum in *Roystonea regia*. The increase of index value from young plants to adult plants is highest in *L. rotundifolia* (477.66%), and lowest in *P. reclinata* (20.77%). It may be noted that, in almost all the species, the stomatal index is higher in adult plants except in *P. pusilla*. The per cent increase of index value for adult plants with isolateral leaves (i.e. *P. pusilla* and *P. reclinata*) is either negative or insignificant.

Width of Cells

The width of epidermal cells on both abaxial and adaxial epidermis has been compared in young and adult plants (table 4). The maximum width in both young and adult palms occurs in *S. zalacca* (figures 3D, 4A,B) followed by *Areca triandra* (figure 4C,D) and minimum in *L. rotundifolia* (figure 2A). The adaxial epidermal cells are often wider than abaxial cells in dorsiventral leaves : *Areca triandra* (figures 4D, 5A), *Caryota urens* (figures 1A, 2B), *Chrysalidocarpus lutescens* (figures 2C, 5B), *Roystonea regia* and *Salacca zalacca* (figures 3D, 4A). A few exceptions occur in the dorsiventral leaves of *L. rotundifolia* and *Veitchia merrillii* (figure 1C, D) where the adaxial epidermal cells are narrower than the abaxial cells. The situation is reverse in the plants with isolateral leaves, e.g. *H. dichotoma*, *P. pusilla* and *P. reclinata*.

The width of epidermal cells becomes significantly smaller in adult *Caryota urens* (figures 1A, 2B), *H. dichotoma* (figures 3B, C), *L. rotundifolia*, *P. pusilla* (figures 5C, D), *P. reclinata* and *S. zalacca* (figures 3D, 4B).

Table 5 shows the *t*-values of various observations and measurements, and their significance at the 1% and 5% levels with d.f. 18. From this table it appears that the differences of stomatal frequencies between adult and young plants on abaxial epidermis are highly significant at the 1% level, and on adaxial epidermis, out of the three species (with isolateral leaves) calculated, two are significant at the 1% level. The difference of length of guard cells between adult and young plants on abaxial epidermis are significant at the 1% level in five species, and at 5% level in one species, and on adaxial epidermis the *t*-values for five species are significant at 1% level. That is, for two species (with isolateral leaves) the *t*-values on both abaxial and adaxial epidermis are significantly negative which indicate that the length of guard cells decrease significantly in adult plants. The *t*-values of width of epidermal cells are significant at the 1% level in six species on abaxial and adaxial epidermis, and at 5% level in two species on abaxial epidermis. The significantly negative *t*-values occur in six species on abaxial epidermis, and five species on adaxial epidermis. These findings indicate that the width of epidermal cells decreases in adult plants.

Conclusions and Discussion

The number of stomata per unit area is significantly higher in adult than in young plants. In *Phoenix reclinata* the increase, especially on adaxial epidermis, is phenomenal. Plants with isolateral leaves (e.g. *Hyphaene dichotoma*,

Table 3 *Stomatal indices of young and adult palms (from abaxial epidermis)*

Species	Young plants (A)	Adult Plants (B)	B–A	% increase
Livistona rotundifolia	2.73	15.77	13.04	477.66
Phoenix pusilla	5.14	4.54	—0.60	—11.67
P. reclinata	5.20	6.28	1.08	20.77
Hyphaene dichotoma	5.81	8.24	2.43	41.82
Salacca zalacca	10.21	15.41	5.20	50.93
Caryota urens	5.07	8.67	3.60	71.01
Areca triandra	5.23	7.88	2.65	50.67
Chrysalidocarpus lutescens	7.88	10.16	2.28	28.93
Roystonea regia	8.31	17.03	8.72	104.93
Veitchia merrillii	6.84	11.69	4.85	70.91

Table 4 *Width of an epidermal cell (μm)**

Species	Young plants		Adult plants	
	Abaxial epidermis	Adaxial epidermis	Abaxial epidermis	Adaxial epidermis
Livistona rotundifolia	8.1±0.21	6.7±0.18	5.9±0.16	5.0±0.03
Phoenix pusilla	10.9±0.20	10.6±0.22	8.5±0.20	8.5±0.35
P. reclinata	10.1±0.31	10.0±0.55	6.3±0.28	6.7±0.24
Hyphaene dichotoma	15.4±0.41	15.0±0.37	11.7±0.52	11.3±0.36
Salacca zalacca	28.0±0.97	31.5±0.31	24.9±0.62	28.4±0.73
Caryota urens	16.5±0.38	17.0±0.65	14.5±0.08	15.9±0.39
Areca triandra	19.7±0.87	24.6±1.02	24.8±0.63	27.1±2.02
Chrysalidocarpus lutescens	14.2±0.27	16.5±0.28	13.7±0.27	16.1±0.84
Roystonea regia	12.7±0.44	14.6±0.25	14.2±0.30	16.7±0.51
Veitchia merrillii	21.9±0.52	18.7±0.51	22.0±0.97	18.3±0.93

* Average for 10 observations

Table 5 *Statistical evaluation of the variations of quantitative characteristics of stomata and epidermal cells between young and adult palms*

Species	Stomatal frequency				Length of guard cells				Width of epidermal cells			
	Abaxial epidermis		Adaxial epidermis		Abaxial epidermis		Adaxial epidermis		Abaxial epidermis		Adaxial epidermis	
	t	Statistical Significance	*t*	Statistical Significance	*t*	Statistical Significance	*t*	Statistical Significance	*t*	Statistical Significance	*t*	Statistical Significance
Livistona rotundifolia	56.428	++	—	—	1.367	—	—	—	—8.485	++	—9.097	++
Phoenix pusilla	4.157	++	1.205	—	1.170	—	3.340	++	—8.485	++	—5.177	++
P. reclinata	16.059	++	24.279	++	—7.004	++	—5.032	++	—9.073	++	—5.633	++
Hyphaene dichotoma	10.477	++	18.820	++	—2.689	+	—1.118	—	—5.527	++	—7.187	++
Salacca zalacca	8.400	++	—	—	9.637	++	6.960	++	—2.701	+	—3.871	++
Caryota urens	6.541	++	—	—	1.286	—	9.751	++	—5.099	++	—1.491	—
Areca triandra	3.785	++	—	—	3.796	++	—	—	4.739	++	1.131	—
Chrysalidocarpus lutescens	8.784	++	—	—	5.698	++	4.569	++	1.152	—	—0.440	—
Roystonea regia	16.384	++	—	—	1.955	—	1.867	—	2.704		3.680	++
Veitchia merrillii	10.104	++	—	—	4.974	++	—	—	0.091	—	—0.453	—

The evaluation of *t*: + significant ($P=0.05$); ++ significant ($P=0.01$)

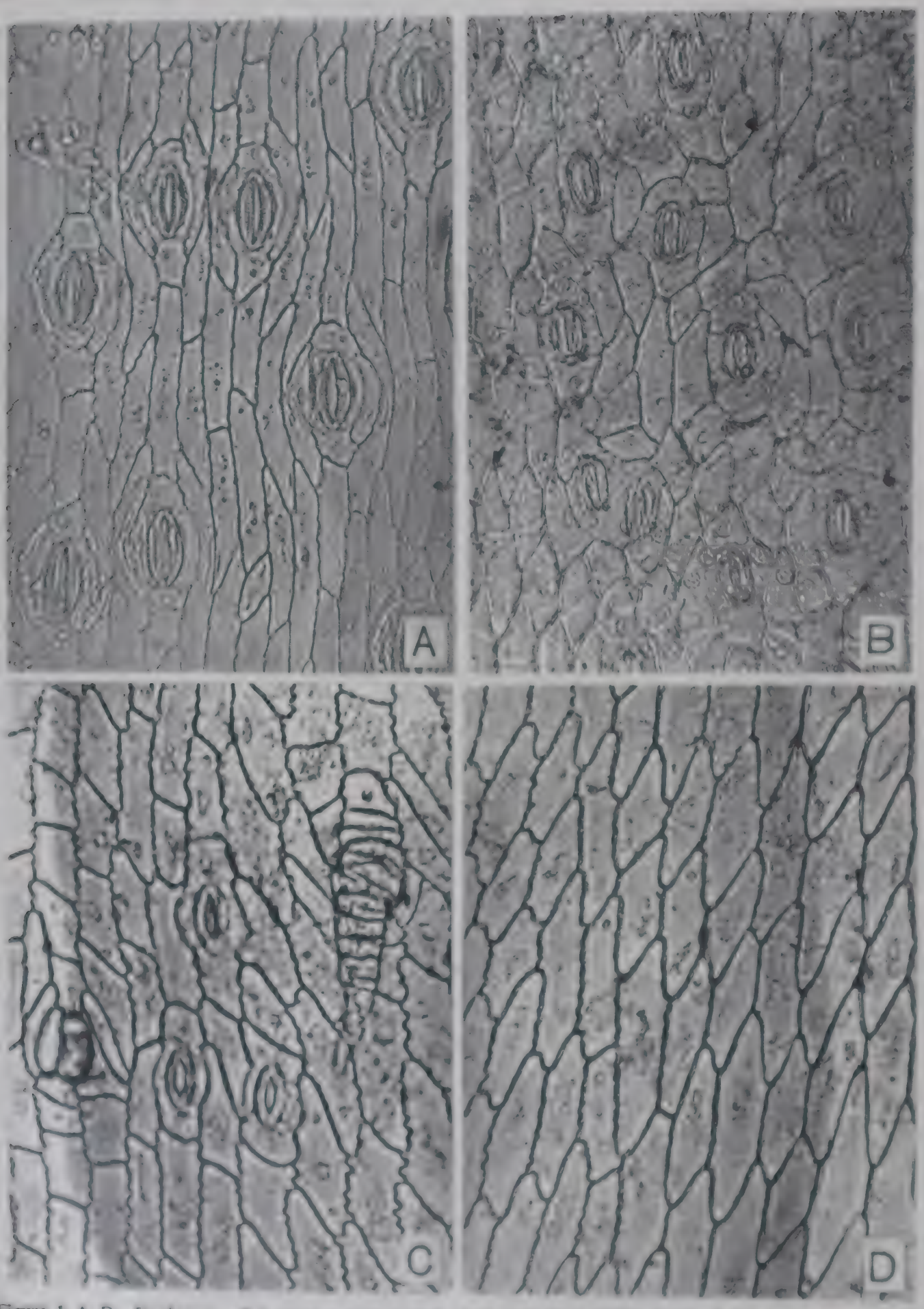


Figure 1 A–D. Epidermis of lamina, surface view. (× 330). A. *Caryota urens*, abaxial epidermis of adult plant; B. *Veitchia merrillii*, abaxial epidermis of adult plant; C. *V. merrillii*, abaxial epidermis of young plant; D. *V. merrillii*, adaxial epidermis of young plant

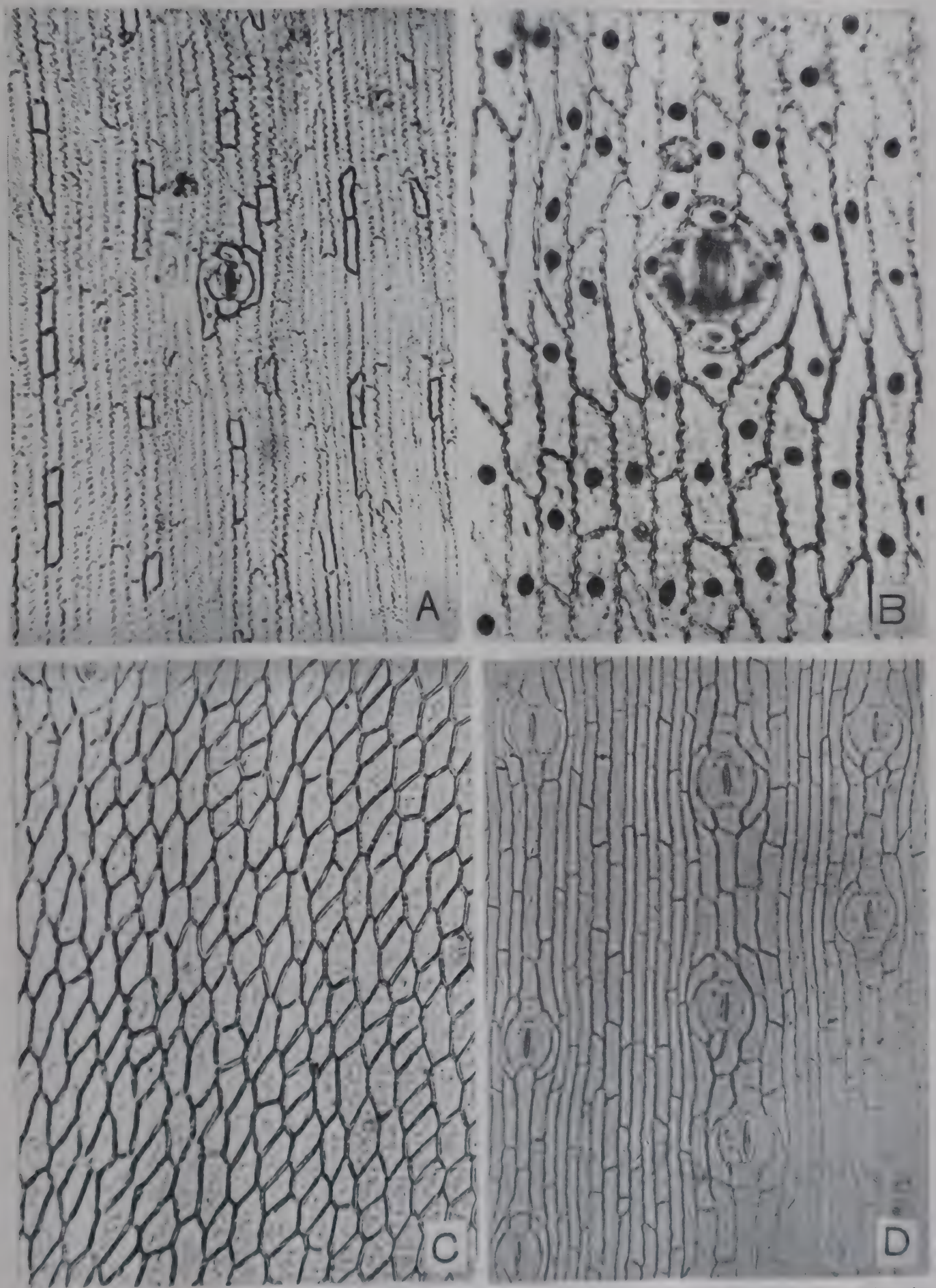


Figure 2 A–D Epidermis of lamina, surface view (× 330); **A**, *Livistona rotundifolia*, adaxial epidermis of young plant; **B**, *Caryota urens*, adaxial epidermis of young plant; **C**, *Chrysalidocarpus lutescens*, adaxial epidermis of adult plant showing a stoma on left side top of the photomicrograph; **D**, *Phoenix reclinata*, abaxial epidermis of young plant

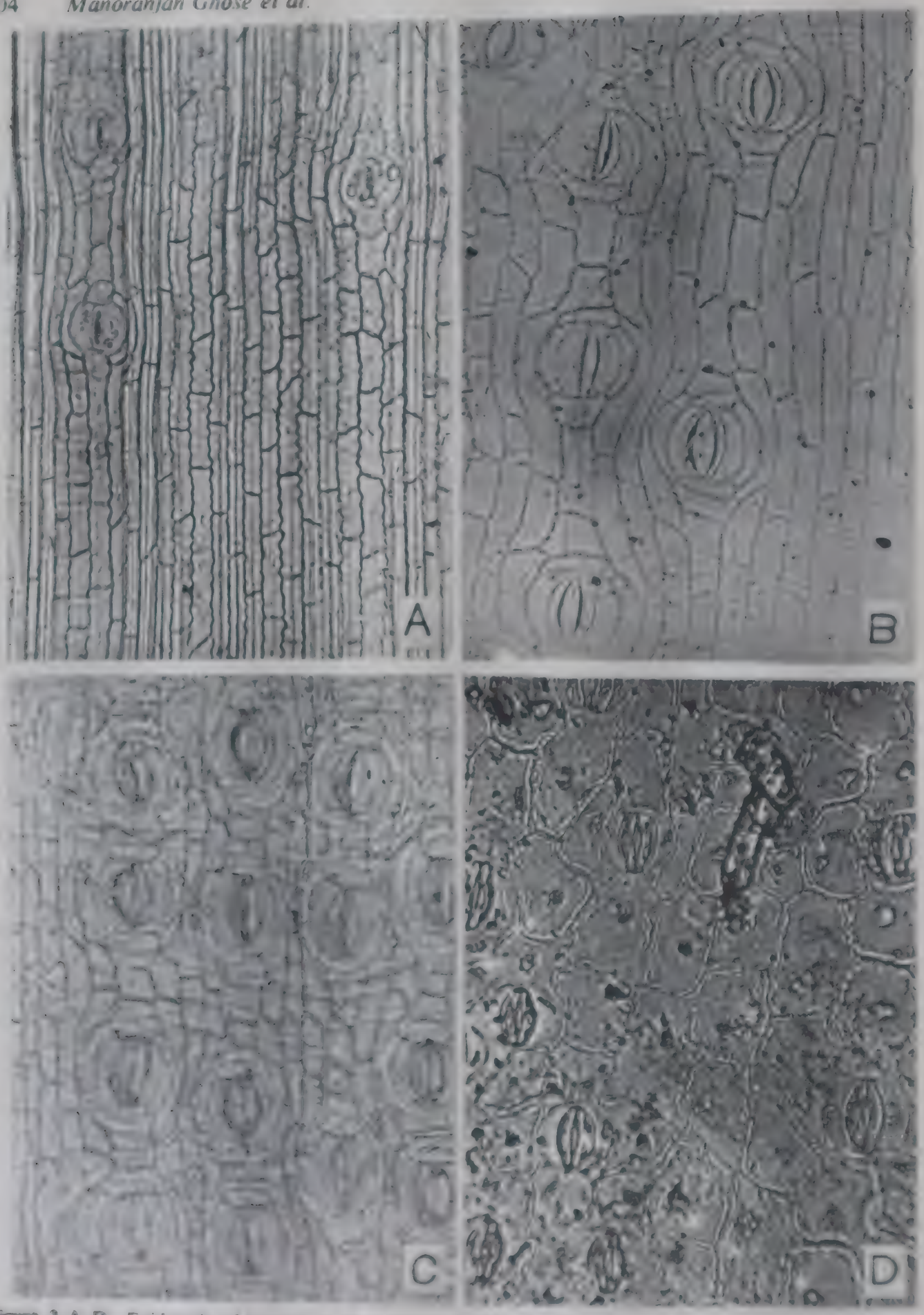


Figure 3 A–D Epidermis of lamina, surface view (× 330): A, *Phoenix reclinata*, adaxial epidermis of young plant; B, *Hyphaene dichotoma*, adaxial epidermis of young plant; C, *H. dichotoma*, adaxial epidermis of adult plant; D, *Salacca zalacca*, abaxial epidermis of young plant

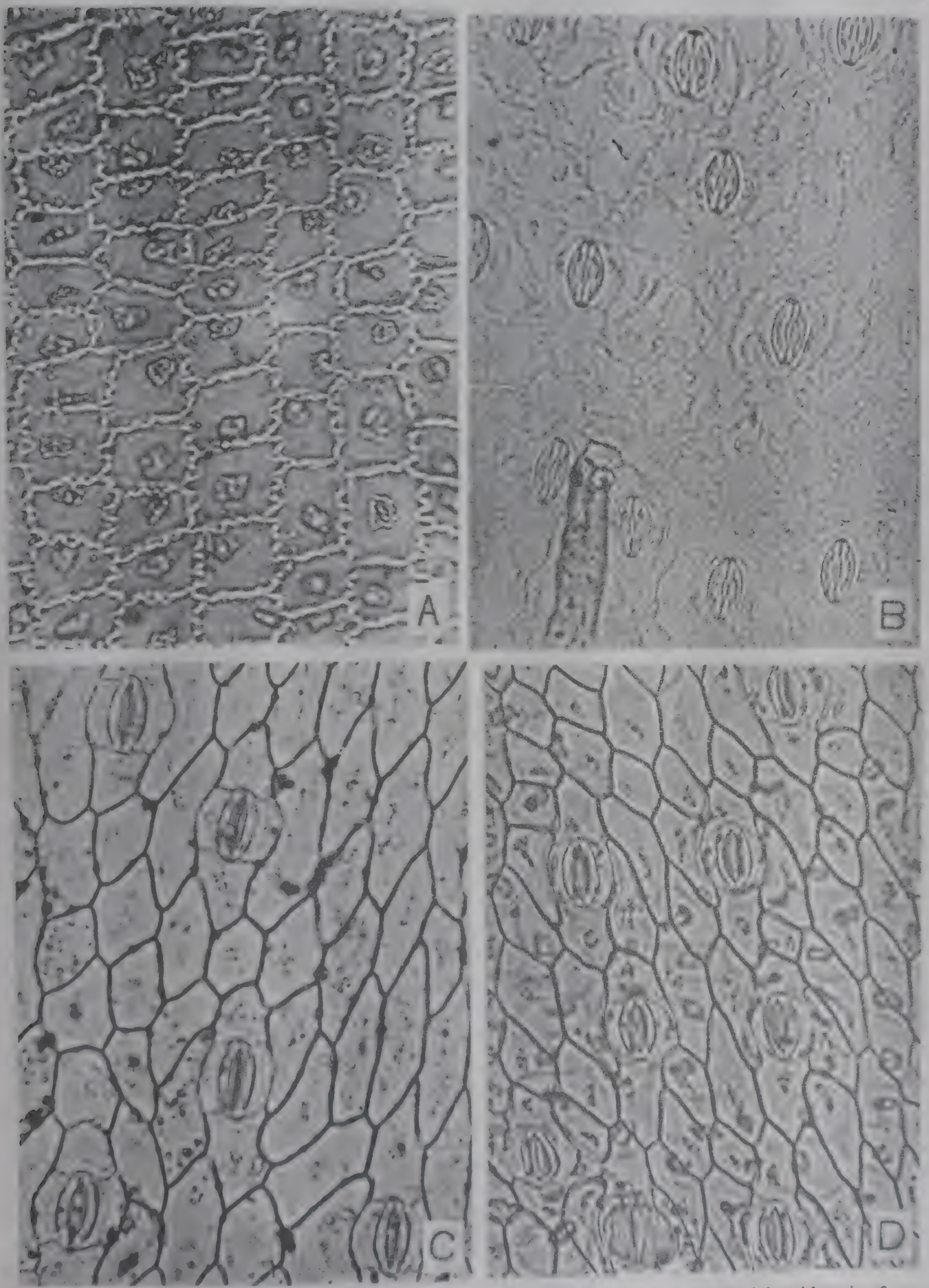


Figure 4 A–D Epidermis of lamina, surface view (× 330); **A**, *Salacca zalacca*, adaxial epidermis of young plant; **B**, *S. zalacca*, abaxial epidermis of adult plant; **C**, *Areca triandra*, abaxial epidermis of young plant; **D**, *A. triandra*, abaxial epidermis of adult plant

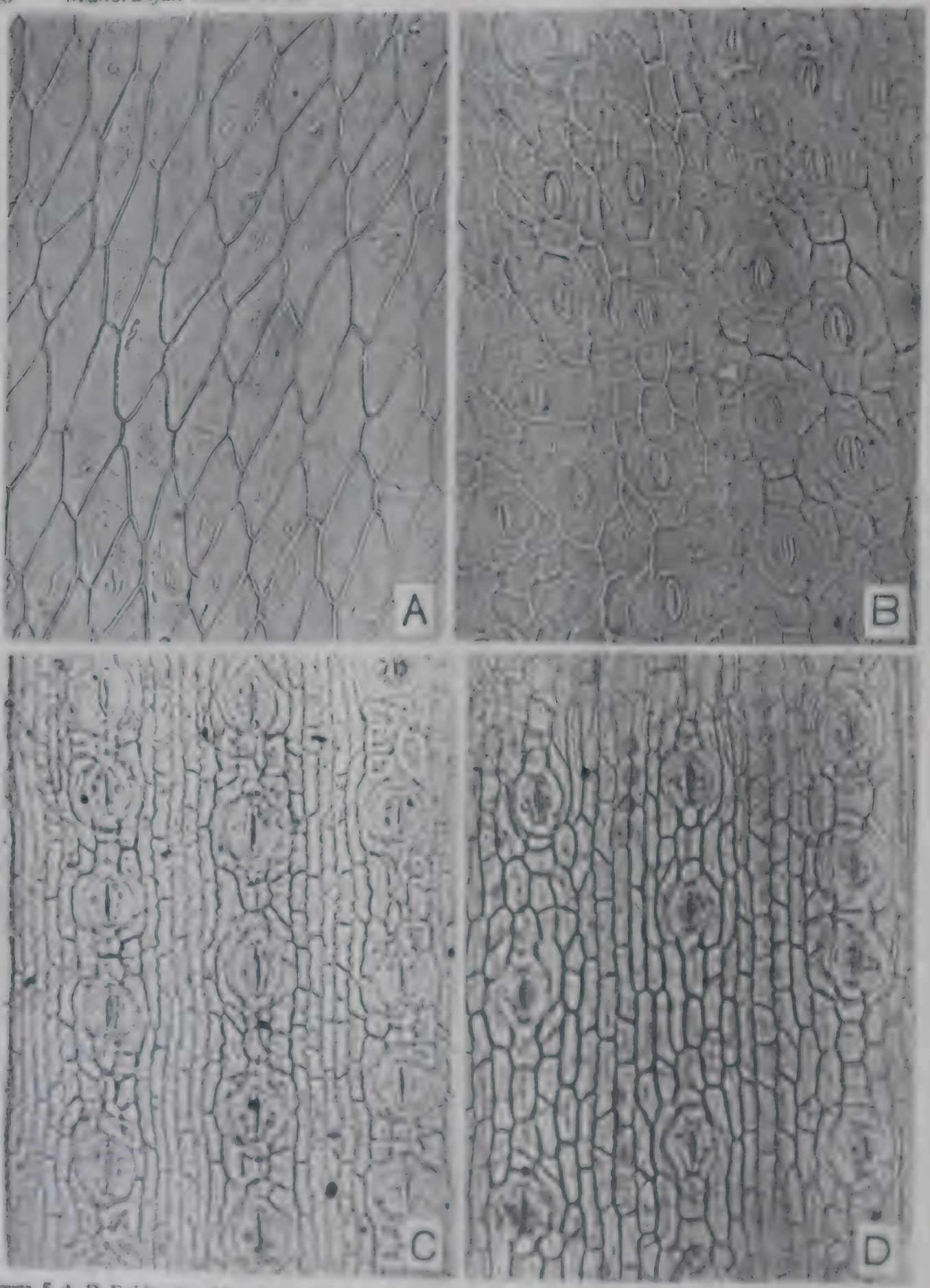


Figure 5 A–D Epidermis of lamina, surface view (× 330): A, *Areca triandra*, adaxial epidermis of adult plant; B, *Chrysalidocarpus lutescens*, abaxial epidermis of adult plant; C, *Phoenix pusilla*, abaxial epidermis of adult plant; D, *P. pusilla*, abaxial epidermis of young plant

Phoenix pusilla and *P. reclinata*) show almost equal or considerable number of stomata on abaxial and adaxial epidermis. On the other hand, plants with dorsiventral leaves (e.g. *Areca*, *Caryota*, *Chrysalidocarpus*, etc.) occasionally have stomata on the adaxial surface. This finding is in conformity with the previous work of Ghose & Davis (1973) on different species of palms. In *Livistona rotundifolia* the frequency of stomata increases several-fold from young to adult plants which indicates that mass transformation of epidermal cells into guard cells takes place at a later stage of growth. A similar situation was also reported in *Livistona chinensis* by Ghose and Davis (1973).

It is evident from tables 1 and 5 that there are two negative associations: (*a*) between the stomatal frequency and the size (length) of stomata (e.g. *Phoenix reclinata* and *Hyphaene dichotoma*), and (*b*) between the stomatal frequency and the width of epidermal cells (e.g. *Livistona*, *Phoenix pusilla*, *P. reclinata*, *Hyphaene*, *Salacca* and *Caryota*). In other plants, particularly in the arecoid major group, these negative associations do not occur. Negative linear correlation between the frequency and length of guard cells have been reported by Kutik (1973), Salisbury (1927), Slavik (1963), Miskin and Rasmusson (1970), and others. Similarly, the negative linear correlation between the frequency of stomata and the area of epidermal cells were reported by Kutik (1973), Pazourek (1965), Salisbury (1927), and others. These correlations resulted from their investigations only in adult members of different taxa other than the palms.

The stomatal index varies significantly in the young and adult plants with dorsiventral leaves, and the variation is insignificant or negative in those with isolateral leaves. The width of epidermal cells also decreases significantly in adult plants. As the width of cells decreases in adult plants, the number of cells per unit area increases, and this increase might be more in plants with isolateral leaves than the concomitant increase in the number of stomata which lowers the index value.

The sequence of stomatal frequency in table 1 can be compared with the sequence of species according to Moore's (1973) classification of palms. The lowest frequencies (in adult plants) occur in species of arecoid and caryotoid groups, while the highest frequencies occur in species of coryphoid and phoenicoid groups. There is insignificant resemblance between Moore's (1973) systematic sequence of evolutionary lines and major groups, and the sequence based on stomatal frequency. There is considerable variation among the species of individual major groups in frequency of stomata. For example, within the arecoid major group, *Chrysalidocarpus lutescens* shows the highest frequency, and *Areca triandra* the lowest. Similarly, within the phoenicoid major group, the highest frequency occurs in *Phoenix reclinata* and lowest in *P. pusilla*.

References

Basu S K and Basu S 1978 Epidermal studies in eophylls (juvenile leaves) of some arecoid palms; *Bull. Bot. Sur. India* **20** 124–132

Bavappa K V A 1966 Morphological and anatomical studies in *Areca catechu* Linn. and *A. triandra* Roxb; *Phytomorphology* **16** 436–443

Ghose M and Davis T A 1973 Stomata and trichomes in leaves of young and adult palms; *Phytomorphology* **23** 216–229

Kutik J 1973 The relationships between quantitative characteristics of stomata and epidermal cells of leaf epidermis; *Biol. Plant.* **15** 324–328

Mahabale T S 1982 *Palms of India* (Pune: Maharashtra Association for the Cultivation of Science, Research Institute)

—— and Shirke N 1967 The genus *Caryota* in India; *J. Bombay nat. Hist. Soc.* **64** 462–487

Miskin K E and Rasmusson D C 1970 Frequency and distribution of stomata in barley; *Crop. Sci.* **10** 575–578

Moore Jr H E 1973 The major groups of palms and their distribution; *Gentes Herb.* **11** 27–141

Pazourek J 1965 The symmetry of the lateral leaflets of *Medicago sativa* L.; *Biol. Plant.* **7** 261–269

Salisbury E J 1927 On the causes and ecological significance of stomatal frequency with special reference to the woodland flora; *Phyl. Trans. R. Soc. Ser.* **B 216** 1–65

Slavik B 1963 The distribution of transpiration rate, water saturation deficit, stomata number and size, photosynthetic and respiration rate in the area of the tobacco leaf blade; *Biol. Plant.* **5** 143–153

Tomlinson P B 1961 *Anatomy of the Monocotyledons*. Vol. II *Palmae* (London: Oxford University Press)

Trivedi B S and Upadhyay N 1979 Epidermal structures in Palmae; *Biol. Mem.* **4** 85–117

Proc. Indian natn. Sci. Acad. **B51** No. 5 pp 609-626 (1985)

Cytological Investigations on some Grasses from Punjab Plain, North India

S S BIR and MANJU SAHNI
Department of Botany, Punjabi University, Patiala 147002

(Received 2 April 1984; after revision 19 September 1985)

Chromosomal analysis of 22 grass species (31 taxa) from Punjab plain revealed that: (*i*) In *Saccharum bengalense*, the hexaploids with n=30 are very common (in over 50% populations). Tetraploids with n=20 are comparatively less common and diploids with n=10 or 11 are rare. Cytomixis is noticed in hexaploid and tetraploid plants; (*ii*) In *S. spontaneum* hexaploid individuals with aneuploid number n=27 are abundant. Plants with n=29 (aneuploid) show multinucleate pollen mother cells; (*iii*) New chromosome numbers are recorded for 10 species.

Nearly 65% taxa are polyploids. The great genetical variability of grasses as evident from large scale variation in chromosome numbers seems to be due to eu- and aneu-ploidy as well as cytomixis and formation of multinucleate pollen mother cells. As compared to dicot plants the vegetative propagation and apomixis so common in grasses, account for the stabilization of genetically variable populations.

Key Words: Chromosome numbers, Grasses, Gramineae, Cytomixis, Multinucleate PMCs

Introduction

Although on world-wide basis a lot of work has been done on the cytology of grasses yet from India regional analysis of grass flora is essential in view of great variability in vegetation under different climatic regions. Further, the biosystematic studies are expected to contribute towards the taxonomy of the members of the group in India.

Chromosomal studies on 31 grass taxa from Punjab plain were made from the view point of improvement of food and fodder resources. Earlier, information on North and East Indian grasses was provided by Mehra et al. (1968), Mehra and Sunder (1970) and Mehra (1982). The reinvestigation of more populations of some taxa has been helpful in confirmation of the earlier reported chromosome numbers as well as in bringing out the variability for which grasses are now well known.

Two species, viz., *Saccharum bengalense* and *S. spontaneum* which are of tremendous economic importance and have a number of biotypes were subjected to thorough analysis.

Materials and Methods

Wild plants growing in and around the cities of Patiala and Ropar in the Punjab State, were subjected to analysis (see table 1*). Usual acetocarmine squash techniques were applied. The arrangement of various genera into different tribes is based on Bor (1960) with species being arranged alphabetically.

Observations

Meiotic divisions in all the taxa were of successive type with the orientation of spindle in the second division mostly being at right angle to the first. The second division in the two nuclei may or may not be synchronous. By and large, the course of meiosis was perfectly normal except for few species. Information about the studied taxa and populations if any, recorded chromosome numbers and ploidy level is provided in table 1 which also incorporates data on voucher specimens and previous reports from India. Features of interest are as follows:

Saccharum (x=10)*

Its two species, *S. bengalense* and *S. spontaneum* are very widely spread perennial grasses of warmer parts of North India.

(*a*) *Saccharum bengalense*

The eighteen populations studied, revealed the existence of four cytotypes:

Cytotype-α (n=10): Young spikelets of population no. 4 invariably showed the presence of 10 bivalents at diakinesis and M-I (figure 1). At M-I, there was often a tendency towards early disjunction of few bivalents. One of the bivalents was of comparatively large size. Regular distribution of 10+10 chromosomes at A-I was noticed. This population is diploid.

Cytotype-β (n=11) Both the populations showed an aneuploid number at diploid level. In population no. 16 meiosis was characterized by the presence of 11_{II} at diakinesis with one bivalent showing early disjunction. In population no. 5 nearly about 50% of the observed PMCs showed formation of 11_{II} at diakinesis and M-I. At mixed A-I, 22 chromosomes were clearly seen (figure 2); whereas in the rest of the 50% PMCs, M-I was characterized by the presence of 2 or 4_I. At A-I, the migration of chromosomes to the poles was irregular with 3 chromosomes being left near the centre of the spindle while 8 and 11 chromosomes were present at the two poles. At T-I, these chromosomes were visible as laggards. The meiosis revealed quite a large number of irregularities; for example, at A-I, 10+12 distribution or the presence of 2 laggards was seen. There was frequent formation of monads, dyads, triads, tetrads and polyads. The micronuclei were commonly present. Pollen with variable genetical constitution was the final result. An analysis of microsporogenesis is given in table 2 which speaks for the high incidence of monads with and without micronuclei showing that there was quite frequent complete failure of meiotic divisions and alongside formation of restitution nucleus. As a consequence of all these meiotic irregularities, the pollen fertility was reduced to about 80%. Thus-formed pollen with variable genetic constitution, if viable, ultimately lead to populations with variable chromosome numbers.

*Vouchers deposited in PUN. Basic numbers mentioned in table 1 are all those which have been recorded or postulated so far whereas those mentioned in the text after genus are those numbers on which the presently studied taxa are considered to be based and these form the basis of calculation of their ploidy levels

Table 1 *Chromosome numbers in certain grass taxa* of Punjab plain*

S.No.	Name of taxa	Locality	Chromosome number	Ploidy level	**Previous reports from India
1	2	3	4	5	6
	Tribe : Andropogoneae				
	SACCHARUM Linn. (x=10)				
1.	(*a*) *S. bengalense* Retz. (Population no. 4)	Patiala and its environos 250m	n=10 (Fig. 1)	Diploid	2n=20, 22, 24, 40, 64 (Mehra et al. 1968, Mehra & Kalia 1973)
	(*b*) *S. bengalense* Retz. (Population nos. 5 & 16)	Patiala and its environs	n=11 (Fig. 2)	Diploid (aneuploid)	
	(*c*) *S. bengalense* Retz. (Population nos. 2, 3, 12, 13 and 14)	Patiala and its environs	n=20 (Figs. 3-4)	Tetraploid	
	(*d*) *S. bengalense* Retz. (Population nos. 1, 6, 7, 8, 9, 10, 11, 15, 17 and 18)	Patiala and its environs	n=30 (Fig. 5)	Hexaploid	
2.	(*a*) *S. spontaneum* Linn. (Population nos. 4 and 5)	Ropar 325m	n=20 (Fig. 6)	Tetraploid	2n=68 (Kuwada 1915) 2n=64 (Dutt & Rao 1933) 2n=54–128 (Singh 1934) 2n=96 (Janaki-Ammal & Singh 1936)
	(*b*) *S. spontaneum* Linn. (Population nos. 1, 2, 6, 7, 8, 9, 10, 12, 13, 14, 16, and 17)	Ropar 325 m and Patiala (250 m)	n=27 (Figs. 7-8)	Hexaploid (aneuploid)	2n=112 (Janaki-Ammal 1941, Nair 1972) 2n=128 (Rao & Babu 1955) 2n=120 (Kandaswami & Rao 1957) 2n=40–128 & 64–120 (Panje & Babu 1968) 2n=64–120 (Jagathensan & Sreenivasan 1960) 2n=58–60 (Sinha & Jha 1972)
	(*c*) *S. spontaneum* Linn. (Population no. 11)	Near Sirsa river	n=28 (Fig. 10)	Hexaploid (aneuploid)	2n=64, 80 (Nair 1973) 2n=40-46, 54, 56, 72, 72+4f (Mehra & Sood 1974)
	(*d*) *S. spontaneum* Linn. (Population nos. 3 and 15)	Ropar and its environs	n=29 (Fig. 11)	Hexaploid (aneuploid)	
	ISCHAEMUM Linn. (x=9, 10)				
3.	*I. rugosum* Salisb.	Punjabi University campus 250m	n=20	Tetraploid	2n=20 (Singh & Godward 1960) 2n=20, 40, 60 (Oke 1971) 2n=44 (Sindhe & Narayan 1976)

1	2	3	4	5	6
	HETEROPOGON Pers. (x=10, 11)				
4.	*H. contortus* (Linn.) Roem Schult	Baradari Garden Patiala	n=29+	Hexaploid (aneuploid)	2n=20 (Janaki-Ammal 1945) 2n=60, 80, 80–88 (Mehra 1954) 2n=40 (Singh 1964, Mehra 1982) 2n=50 (Roy et al. 1965) 2n=40, 60 (Mehra et al. 1968, Sinha & Jha 1972) 2n=40. 50, 60 (Gupta 1971)
	PANICUM Linn. (x=7, 8, 9, 10)				
5.	*P. antiodotale* Retz.	Bahadurgarh Patiala 250 m	n=14+	Tetraploid	2n=18, 36 (Narayan 1962) 2n=18 (Jauhar & Joshi 1966, Sinha & Jha 1972) 2n=36 (Mehra et al. 1968)
6.	*P. maximum* Jacq.	Punjabi Bagh Patiala	n=27+ (Fig. 13)	Hexaploid	2n=36 (Janakk-Ammal 1945, Raman et al. 1959, Raman & Krishnaswamy 1959) 2n=32 (Chandola 1959, Jauhar & Joshi 1969) 2n=18, 32 (Jauhar 1969, Nath, Swaminathan & Mehra 1970) 2n=32, 36 (Sinha & Jha 1972) 2n=42 (Khosla & Mehra 1973).
	SETARIA Beauv. (x=9)				
7.	*S. tomentosa* (Roxb.) Kunth	Baradari Gardens Patiala	n=19+ (Fig. 14)	Tetraploid (aneuploid)	2n=34 (Mehra 1982), 2n=36 (Gupta 1963, 1969, 1971, Mitra & Datta 1967, Malik & Tripathi 1970, Saxena & Gupta 1970, Gupta & Yashvir 1971, Khosla & Sharma 1973, Mehra & Remanandan 1973, Nagabhushana & Sindhe 1977).
8.	*S. verticillata* (Linn.) P. Beauv.	Punjabi University Patiala	n=27	Hexaploid	2n=36 (Krishnaswamy & Ayyanagar 1935, Krinaswamy & Raman 1949, Krishnaswamy 1951, Sharma & De 1956, Chandola 1959) 2n=54 (Raman et al. 1959, Gupta & Yashvir 1971, Khosla 1972, Khosla & Sharma 1973) 2n=18, 36, 54 (Mehra et al. 1968)

1	2	3	4	5	6
	ECHINOCHLOA P. Beauv. (x=9)				
9.	*E. crusgalli* (Linn.) P. Beauv. Var. *Crusgalli*	Sadhubella near Punjabi University	n=25+	Hexaploid (aneuploid)	2n=48 (Singh & Godward 1960) 2n=54 (Malik & Mary 1970, Malik & Tripathi 1974, Mehra 1982)
	UROCHLOA Stapf (x=7, 8, 9, 15)				
10.	*U. panicoides* P. Beauv.	Bahadurgarh Patiala	n=24	Hexaploid	2n=48 (Raman et al. 1959, Mitra & Datta 1967) 2n=36 (Mehra et al. 1968, Saxena & Gupta 1970) 2n=46 (Malik & Mary 1970)
	BRACHIARIA Griseb. (x=7, 9, 10)				
11.	*B. distachya* (Linn.) Stapf	Punjabi Bagh, Patiala	n=10+	Diploid	2n=72 (Mehra & Chaudhary 1976) 2n=36 (Muniyamma 1976, Sharma & Sukhjit 1980, Geepi & Muniyamma 1981)
	DIGITARIA Heist ex Fabr. (x=9, 15, 17)				
12.	*D. adscendens* (H.B.K.) Henr.	Punjabi University Patiala	n=36	Octoploid	2n=36 (Krishnaswamy 1940, Nath & Swaminathan 1957, Nath, Swaminathan & Mehra 1970) 2n=60 (Mullay & Leelma (1956) 2n=54 (Malik & Mary 1970, Saxena & Gupta 1970, Muniyamma, Nagabhushana, Sindhe & Narayan 1976, Sarkar, Chakraborty, Sha & Das 1976, Mehra 1982) 2n=54, 72 (Gupta 1971)
	CENCHRUS Linn. (x=9, 17)				
13.	*C. ciliaris* Linn.	Punjabi University Patiala	n=18	Tetraploid	2n=34 (E.K.J. in Darlington & Wylie 1955) 2n=44, 52 (Nath & Swaminathan 1957) 2n=56 (Ponniya et al. 1966) 2n=36, 44, 56, 68 (Ramaswamy, Raman & Menon 1969)

1	2	3	4	5	6
					2n=36 (Malik & Tripathi 1969, 1970, Khosla 1972) 2n=36, 44, 52 (Nath, Swaminathan & Mehra 1970) 2n=45 (Shanthana, Narayan & Shukur 1976) 2n=34, 36 (Mehra 1982)
	Tribe: Aristideae *ARISTIDA* Linn. (x=11, 12, 19)				
14.	*A. adscensionis* Linn.	Punjabi University Patiala	n=11	Diploid	2n=22 (Mehra et al. 1968, Malik & Tripathi 1969)
	Tribe: Aveneae *LOPHOCHLOA* Reichb (x=6)				
15.	(*a*) *L. pheloides* (Vill.) Reichb	Punjabi University Patiala	n=6 (Fig. 15)	Diploid	2n=12, 14, 16 (Mehra et al. 1968)
	(*b*) *L. pheloides* (Vill.) Reichb	Punjabi University Patiala	n=13 (Fig. 16)	Tetraploid (aneuploid)	
	Tribe: Eragrosteae *ERAGROSTIS* P. Beauv. (x=9, 10)				
16.	*E. cilianensis* (All.) Vignolo-Lutati (x=9, 10)	Punjabi University Patiala	n=10	Diploid	2n=20, 40 (Singh & Godward 1960) 2n=70 (Mehra et al. 1968) 2n=20 (Malik & Tripathi 1970)
17.	*E. pilosa* (Linn.) P. Beauv.	Punjabi University Patiala	n=18+	Tetraploid	2n=40 (Mehra et al. 1968)
18.	*E. tremula* Hochst. ex Steud.	Baradari Gardens Patiala	n=14+	Diploid (aneuploid)	2n=20 (Mullay & Leelama 1956, Cristopher & Abraham 1974)
	DACTYLOCTENIUM Willd. (x=9, 10, 12)				
19.	(*a*) *D. aegyptium* (Linn.) P. Beauv.	Punjabi University Patiala	n=9+	Diploid	2n=34 (Krishnaswamy 1931, Krishnaswamy & Ayyanagar 1935) 2n=48 (Janaki-Ammal 1945, Gupta 1971, Christopher & Abraham 1974)
	(*b*) *D. aegyptium* (Linn.) P. Beauv.	Punjabi University Patiala	n=10	Diploid	2n=36 (Chandola 1959) 2n=20, 40 (Mehra et al. 1968)

1	2	3	4	5
(c) *D. aegyptium* (Linn.) P. Beauv.	Punjabi Bagh Patiala	n=19	Tetraploid (aneuploid)	2n=40 (Gupta & Yashvir 1971, Mehra & Sharma 1975, Sharma & Sharma 1978, Mehra 1982) 2n=46 (Mehra & Chaudhary 1974, Mehra 1982) 2n=38 (Sharma & Salam 1980)
LEPTOCHLOA P. Beauv. (x=10)				
20. *L. panicea* (Retz.) Ohwi	Punjabi University Patiala	n=10	Diploid	2n=20 (Mehra et al. 1968) 2n=20+0-1B (Sharma & Parveen 1980)
Tribe: Sporoboleae *Sporobolus* R.Br. Hoshst. x=9, 10, 12				
21. *S. coromandelianus* Retz. Kunth	Punjabi University Patiala	n=18	Tetraploid	2n=36 (Narayan & Muniyamma 1972) 2n=24 (Christopher & Abraham 1974)
22. *S. marginatus* Hochst. ex A. Rich.	Sadhubela near Punjabi University	n=9+	Diploid	2n=36 (Mehra et al. 1968)

*Habit classification of studied grasses S.Nos. 1, 2, 4, 5, 6, 13, 22=Perennials and S.Nos. 3, 7, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21=Annuals

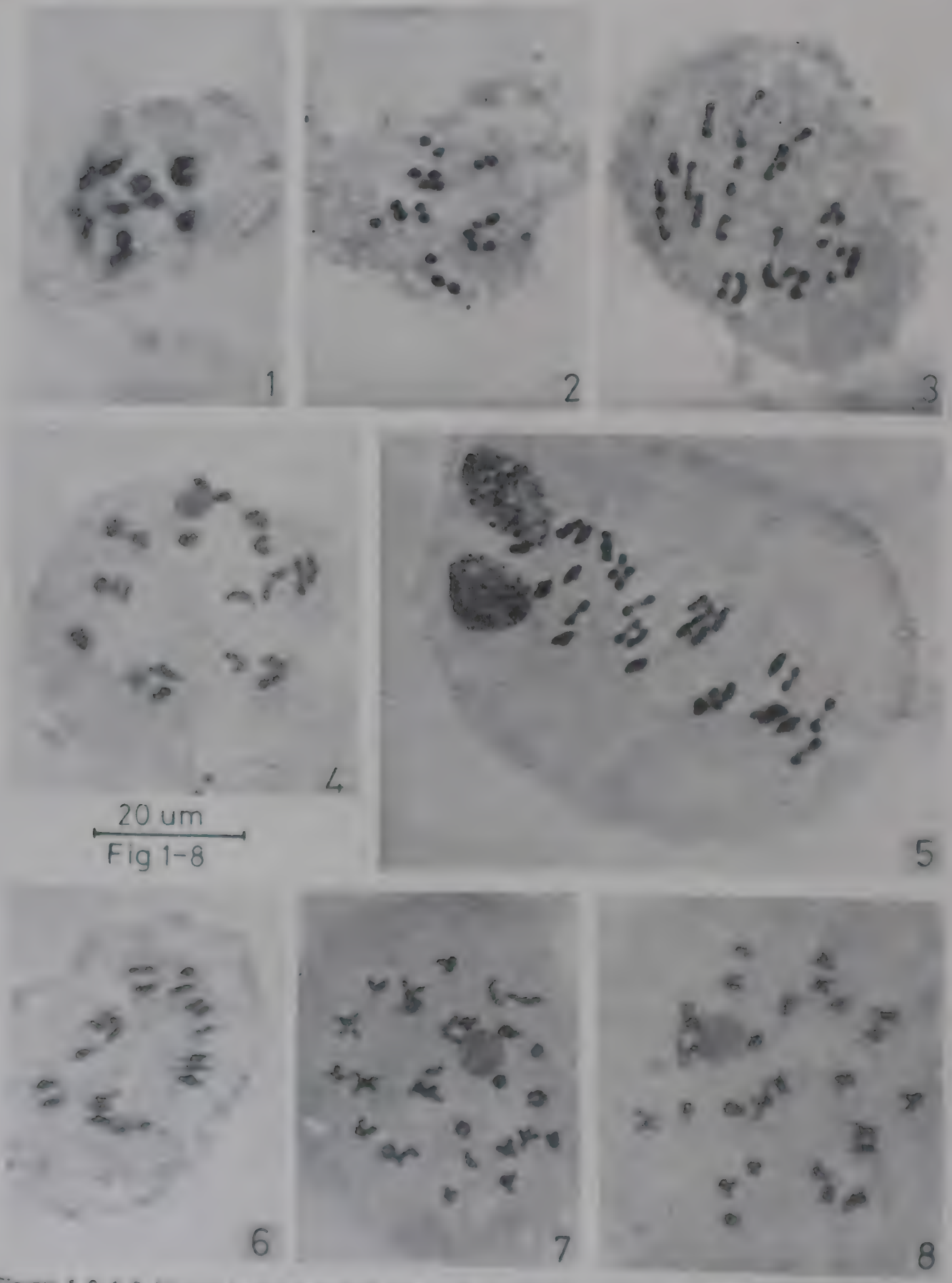
**Information on previous chromosome number reports compiled from Darlington & Wylie (1955), Löve & Löve (1961, 1974 & 1975), Fedorov (1969), Index to Plant Chromosome Numbers (1956 onwards), IOPB chromosome number reports as published in Taxon from 1965 onwards and selected references from Biological abstracts (1970 onwards). Therefore, references to workers mentioned under column on previous reports, are excluded at the end and the reader is referred to the above mentioned works. Irrespective of the fact whether 'n' or 2n chromosome numbers are recorded by previous workers, we have invariably mentioned the '2n' number.

+New cytotypes. The recorded chromosome numbers are quite often indicative of the secondarily evolved new basic number/s through eu- and/or aneuploidy

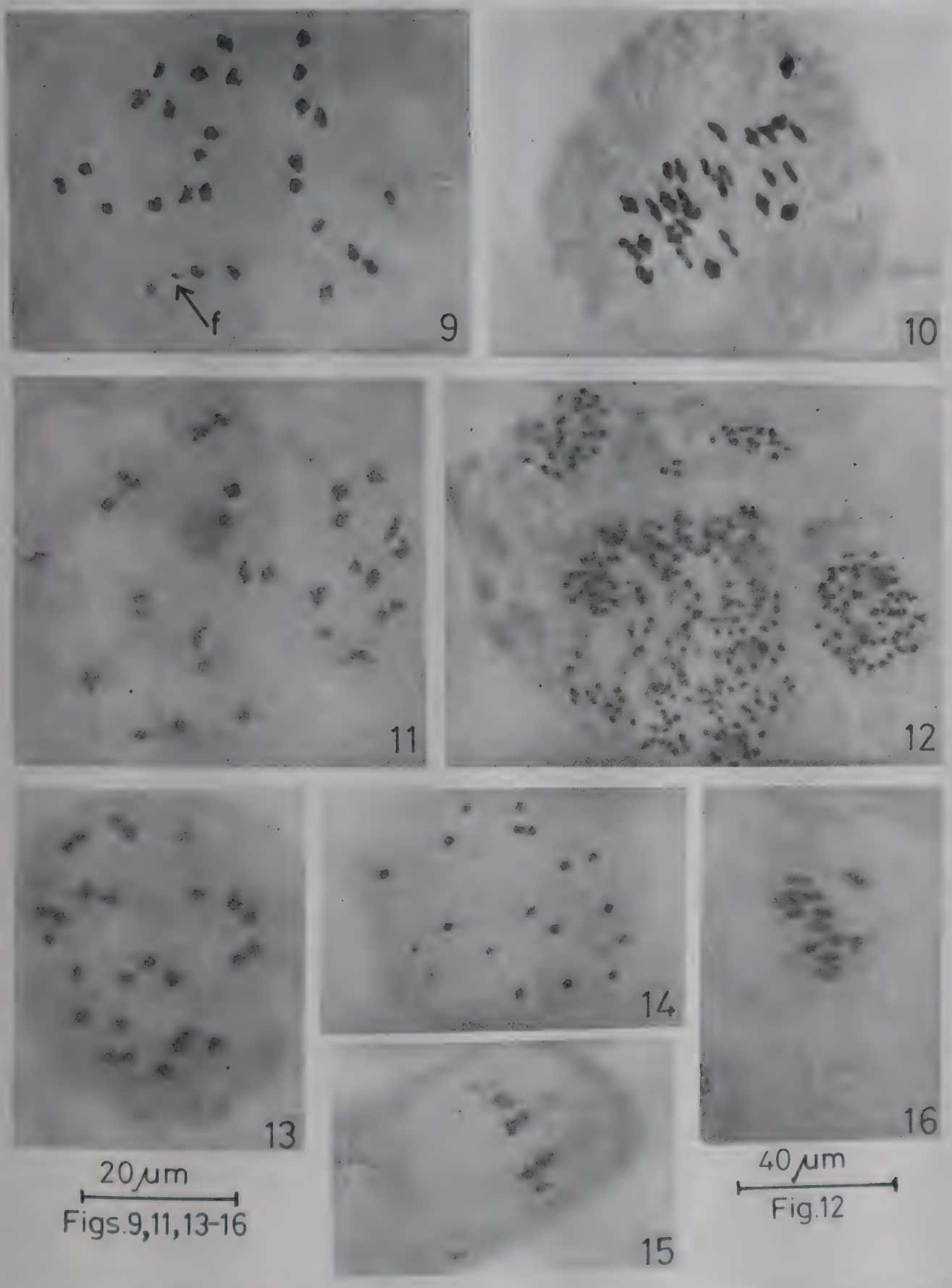
Cytotype-γ (n=20): In four tetraploid populations (nos. 3, 12, 13 and 14) at diakinesis and M-I, 20_{II} were observed (figures 3 & 4). During M-II, 20 chromosomes were clearly discernible at each pole confirming the chromosome number to be n=20.

In population no. 2, cytomixis was observable at different stages of meiosis. There was frequent transfer of chromatin material through cytoplasmic channels. During early stages of meiosis the phenomenon was noticed in only about 20% of the observed PMCs but it was more frequent during the latter stages. Two to five PMCs are usually involved.

Cytotype-δ (n=30): Out of 18 populations studied 10 populations (nos.1, 6–11, 15, 17, 18) showed n=30 indicating that the hexaploid plants are very common in the region. In population nos. 1, 7, 8, 9, 15, 17 and 18 at diakinesis and M-I, 30_{II} were observed (figure 5). At mixed M-II, 60 chromosomes were easily disernible. Meiosis was perfectly



Figures 1–8. 1–5, Meiosis in pollen mother cells of *Saccharum bengalense*. 1, n = 10 at diakinesis. 2, n = 11 at A–I. 3, n = 20 at late M–I. 4, n = 20 at diakinesis. 5, n = 30 at M–I with two nucleoli. 6–8, *S. spontaneum*: 6, n = 20 at M–I; 7–8, n = 20 at diakinesis



Figures 9–16. Meiosis in PMCs: **9–12,** S. *spontaneum;* 9, n=27+1 fragment at M–I; **10,** n=28 at M–I; **11,** n=29 at diakinesis; **12,** Multinucleate pollen mother cell; **13,** *Panicum maximum* n=27 at M–I; **14,** *Setaria tomentosa* n=19 at M–I; **15,** *Lophochloa pheloides* n=6 at M–I (Diploid cytotype); **16,** *Lophochloa pheloides* n=13 at M–I (Tetraploid cytotype)

Figures 17–20. Voucher specimens of different cytotypes: **17–18.** *S. bengalense*, hexaploids of 1st and 2nd category. **19–20.** *Lophochloa pheloides*: **19.** n = 6 (ditraploid). **20.** N = 13 (tetraploid).

Table 2 *Analysis of microsporogenesis in* S. bengalense (*Diploid population no.5 with* $n=11$)

Number of PMCs observed	Monad		Dyad		Triad	Tetrad		Polyad
	with micro-nuclei	without micro-nuclei	with micro-nuclei	without micro-nuclei		with micro-nuclei	without micro-nuclei	
12	—	5	—	2	1	—	4	—
10	—	4	—	2	—	—	4	—
10	—	4	1	2	—	2	1	—
10	—	3	—	3	1	—	2	1
10	1	2	3	—	—	—	4	—
9	1	4	1	—	2	—	—	1
9	—	3	—	2	1	3	—	—
8	—	4	—	2	1	—	1	—
8	2	4	2	—	—	—	—	—
8	1	2	1	—	2	1	—	1
7	—	2	—	1	1	—	2	1
7	—	3	—	—	—	—	4	—
5	—	2	—	2	—	—	1	—
5	1	—	2	...	1	—	1	—
4	—	4	—	—	—	—	—	—
Total 122	6	46	10	16	10	6	24	4
Percentage	4.91	37.70	8.19	13.11	8.19	4.91	19.66	3.27

normal in all of these populations. In population no. 6 also, at M-I, 30_{II} were noticed. But in few PMCs even at late A-I, 5_{II} showed non-disjunction due to interlocking of chiasmata and this lead to reduced pollen fertility. In population no 11, besides the normal 30_{II} the phenomenon of cytomixis was observed in some of the PMCs although only during T-I stages. Here, 2–5 PMCs were involved in formation of communicating channels. In population no. 10, also with n=30 in some PMCs, the two nucleoli were quite persistent even at M-I stage.

Morphological Characteristies of Different Cytotypes of *S. bengalense*

The different cytotypes were clearly discernible though on the basis of microcharacters (cf. table 3). Remarkably the diploid cytotypes-α (n=10) and β (n=11) showed hairs on the upper side of the leaf midrib-whereas these hairs were absent in the tetraploid cytotype-γ (n=20) and the hexaploid cytotype-δ (n=30). The length of the inflorescence was markedly variable in the hexaploid individuals but the diploids with n=10 and n=11 as well as the tetraploids (n=20) exhibited very little size variability.

In the field the various cytotypes could be distinguished by the fact that in the diploids (n=10, 11) the upper palea was produced into a long awn-like process, whereas, this was never the case in the tetraploid (n=20) and hexaploid (n=30) plants which could further be segregated on the basis of the presence of reddish tinge on the fresh leaf sheath in the tetraploid plants and the absence of such a reddish tinge in the hexaploids.

Interestingly, there was lot of variation amongst the hexaploids also. Plants of 10 populations could be segregated into three categories on the basis of characters given in table 4 (see also figures 17 & 18).

Table 3 *Distinguishing characters of various cytotypes in* Saccharum bengalense *from Punjab Plain*

S. No.	Characters	Cytotype-α (n = 10, 2x)	Cytotype-β (n = 11, 2x)	Cytotype-γ (n = 20, 4x)	Cytotype-δ+ (n = 30, 6x)
1.	Culms	Stout	Stout	Less stout	Less stout
2.	Height	2–3 m	3–3.5 m	2–3.5 m	2–3 m
3.	Leaves*				
	(*a*) Length	29–32 cm	35–37 cm	26–30 cm	32–40 cm
	(*b*) Breadth	5–6 mm	6–7 mm	3–4 mm	4–9 mm
	(*c*) Sheath	Reddish tinge present when young	Reddish tinge present	Reddish tinge present	No reddish tinge
4.	Hairy characters of midrib	Hairs on the upper side of midrib	Hairs on the upper side of midrib	Hairs absent on the upper side of midrib	Hairs absent on the upper side of midrib
5.	Length of the inflorescence	42–45 cm	30–32 cm	30–31 cm	18–40 cm
6.	Pollen size**	26–30 × 26–30 μm	28–36 × 26–36 μm	28–38 × 26–36 μm	28–44 × 26–36 μm
7.	Pollen fertility	98.00%	84.00%	92.00%	99.00%

* Based on 10 samples
** Based on 50 samples
+Characters based on detailed study in table 4

Table 4 *Differences in hexaploid plants of* Saccharum bengalense *from different populations*

S. No.	Characters	Category I population nos. 7, 8, 10, 17	Category II population nos. 1, 6, 9, 15, 18	Category III population no. 11.
1.	Height	2–3 m	2 m	2.5 m
2.	Leaves*			
	(*a*) Length	32 cm	40 cm	30 cm
	(*b*) Breadth	9 mm	5 mm	3 mm
3.	Inflorescence length	38 cm	40 cm	18 cm
4.	Pollen size**	28–30 × 26–30 μm	28–44 × 26–36 μm	28–36 × 26–30 μm
5.	Pollen fertility	99.20%	95.50%	97.42%

*Based on 10 samples
**Based on 50 samples

(*b*) *Saccharum spontaneum*: The 17 populations studied showed four different cytotypes with chromosome numbers n=20, 27, 28 and 29. Plants with n=20 represented the tetraploids, whereas all others show aneuploid numbers at hexaploid level.

Cytotype-α (n=20): In population nos. 4 and 5 the meiosis was normal with 20_{II} at diakinesis and M-I (figure 6).

Cytotype-β (n=27): In population nos.6, 7, 8, 10, 12, 14 and 17, several PMCs revealed the presence of 27_{II} at diakinesis and M-I (figures 7, 8). At M-II, 27 chromosomes were countable at each pole but in population no.1 alongwith 27_{II}, two nucleoli were present even at M-I and at A-I chromosomal bridges were seen. In population nos.2 and 16, besides normal 27_{II} laggards have also been observed at A-I and T-I in some PMCs. In population no.9 and 13 besides normal 27_{II}, a chromosomal fragment was also present (figure 9) which disappeared subsequently.

Amongst all the populations of *S. spontaneum* analysed here, the cytotype with n=27 was the most common in distribution.

Cytotype γ (n=28): In population no. 11, 28_{II} were seen at M-I (figure 10) giving n=28. Some of the PMCs at A-I showed late disjunction of one bivalentand at T-I a few laggards.

Cytotype (n=29): In population no. 15, 29_{II} were seen at diakinesis and M-I (figure 11) but in population no. 3 in few PMCs out of 29_{II}, two bivalents were linked together or at A-I late disjunction of one or two bivalents due to interlocking of chiasmata is noticeable. Interspersed among normal PMCs, multinucleate pollen mother cells were also observed (figure 12). Generally, these are spherical with 2-3 or even more nuclei which undergo meiotic divisions and show variable number of chromosomes. Some nuclei are highly polyploid in constitution as seen from the presence of a very high number of chromosomes (about 150_{II}), while the others exhibit very low number of bivalents as 16, 28, 52 etc. (figure 12).

Morphological Characters of Various Cytotypes in *Saccharum spontaneum*

A close scrutiny of the data in table 5 shows that the tetraploid possessed the maximum height and the broadest leaves as compared to the hexaploid cytotypes. But the hexaploids

Table 5 *Characters of various cytotypes in* Saccharum spontaneum *from Punjab plain*

S. No.	Character	Cytotype-α (n=20, 4x)	Cytotype-β (n=27, 6x aneu.)	Cytotype-γ (n=28, 6x aneu.)	Cytotype-δ (n=29, 6x aneu.)
1.	Culms	Stout	Less Stout	Less stout	Stout
2.	Height	3 m	2–2.5 m	2.5 m	2m
3.	Leaves*				
	(*a*) Length	28.5 cm	32 cm	28 cm	50–55 cm
	(*b*) Breadth	5 mm	3 mm	2 mm	2–3 mm
4.	Length of inflorescence	30–33 cm	25–27 cm	25 cm	21 cm
5.	Pollen size**	28–38 × 28–36 μm	38–52 × 34–40 μm	26–38 × 28–36 μm	40–49 × 42–38 μm
6.	Pollen fertility	91.00%	92.12%	99.00%	99.00%

*Based on 10 samples
**Based on 50 samples

cytotype with n=29 had very long leaves, more than 1½ times of that in other cytotypes. It also distinctly had the largest pollen but the inflorescence size was very small. Pollen fertility was higher in hexaploids with n=28, 29 as compared to the cytotypes with n=20 and n=27. Thus, we find that notably tetraploid and hexaploid cytotypes were separable, but within the hexaploids also plants with different number of chromosomes can be segregated (for details see table 5).

Ischaemum (x=10): In *I. rugosum* which is tetraploid (n=20), quite often a few bivalents showed early disjunction as was the case with diploid cytotype (n=10) of *S. bengalense* referred to earlier. Such chromosomes could easily be mistaken for Is or accessory chromosomes and may lead to inaccurate counting of haploid number.

Heteropogon (x=10): The only worked out species *H. contortus* showed 29_{II}, and is hexaploid but with new aneuploid number. The meiosis was perfectly normal.

Panicum (x=7, 9): *P.antidotale* with n=14 is tetraploid based on X=7 and *P. maximum* showing n=27 (figure 13) is hexaploid, based on x=9. Frequently, the presence of chromosomal bridges at A-I was seen in *P. antidotale* resulting in lower pollen fertility.

Setaria (x=9): *S. tomentosa* (n=19) (figure 14) and *S. verticillata* (n=27) are tetraploid and hexaploid grasses but the former showed aneuploidy.

Echinochloa (x=9): The plant of *E. crusgalli* var. *crusgalli* growing near University Campus clearly showed $25/_{II_3}$. These are considered to be hexaploid (aneuploid number). Earlier reports (cf. table 1) from India indicate the presence of both diploid and hexaploid cytotypes.

Urochloa (x=8): The only worked out species, *U. panicoides* is hexaploid as confirmed through the presence of n=24 in plants from Bhadurgarh, Patiala. Earlier. in addition to hexaploid, tetraploid (n=18) based on x=9 and an aneuploid race of hexaploid (n=23) are on record from India (cf.table 1).

Brachiaria (x=10): In contrast to the earlier records (cf. table 1) of tetraploid and octoploid cytotypes based on x=9, *B. distachya* plants from Patiala clearly showed only 10_{II_5} giving n=10. This is a diploid race based on X=10.

Digitaria (x=9): *D. adscendens* shows n=36. The plants are octoploid. Earlier, diploid, tetraploid, haxaploid and octoploid plants are known (cf. table 1). Evidently this grass is cytologically very variable. Characteristic interbivalent connections or loose association of several chromosomes were frequently seen. This resulted in pollen sterility of 40%.

Cenchrus (x=9): Presently recorded tetraploid (n=18) individuals of *C. ciliaris* do not always show 100% normal meiosis. In about 10% of observed PMCs laggards are frequently seen at A-I leading to decreased pollen fertility. This grass is cytologically highly variable with euploid and aneuploid numbers at diploid, tetraploid and hexaploid levels (cf. table 1).

Aristida (x=11): *A. adscensionis* is a diploid (n=11) grass and this confirms the earlier report.

Lophochloa (x=6): Two cytotypes of *L. pheloides* with n=13 (figures 15, 16) were recorded. Comparison of their morphological characters indicates that tetraploid plants are robust in appearance with thicker stem, large leaves, bigger stomata and bigger pollen grains (table 6 and figures 19, 20). We have not come across the additional cytotype with n=7 as recorded by Mehra et al. (1968).

Eragrostis (x=9, 10): Three species namely *E. cilianensis* (n=10), *E. pilosa* (n=18) *E. tremula* (n=14) showed a great cytological

Table 6 *Morphological characteristic of diploid and tetraploid cytotype in* Lophochloa pheloides

S. No.	Characters	Diploid n=6	Tetraploid n=13
1.	Stem		
	Height	20–24 cm	30–38 cm
	Culm	1–2 arising from base	8–12 arising from base
2.	Leaf*		
	(*a*) Sheath length	5.5–6.7 cm	7.5–8.7 cm
	(*b*) Lamina Length	6.5–8.5 cm	8–10 cm
	(*c*) Lamina breadth	3–5 mm	5–8 mm
3.	Stomata**		
	(*a*) Length	27–30 μm	30–35 μm
	(*b*) Breadth	4–8 μm	4–8 μm
	(*c*) Frequency/unit area	2–3	3–4
4.	Inflorescence: Length	5–5.5 cm	8–9 cm
5.	Pollen**		
	(*a*) Size (Length× Breadth)	19-26× 15–23 μm	26–30× 22–30 μm
	(*b*) Fertility	100.00%	100.00%

*Based on 10 samples
**Based on 50 samples

variability. The first one is diploid (x=10), the second one, tetraploid (x=9); and the third one, aneuploid at diploid level or could reflect the existence of another basic number x=7. Earlier reports showed *E. tremula* to be diploid with n=10 (cf. table 1).

Dactyloctenium (x=9): Three cytotypes with n=9, 10 and 19 have been recorded for *D. aegyptium*. Previously, cytotypes with 2n=20, 22, 38, 40 and 46 have been recorded from the North Indian region (cf.table 1). The presently recorded cytotypes are distinguished by microcharacters as given in table 7. The leaves were markedly narrow in cytotype with n=9, which shows comparatively broader stomata with higher frequency per unit area.

Leptochloa (x=10): *L. panicea*, as worked out from University Campus, showed n=10. It is diploid. In spite of a wide sampling, we have not come across any B-Chr. as was reported by Sharma and Parveen (1980).

Sporobolus (x=9): *S. marginatus* (n=9) and *S. coromandelianus* (n=18) are diploid and tetraploid species. For the former, diploid is recorded for the first time from India.

Discussion

Haploid chromosome numbers, in the presently studied grass species varied from n=6 (*Lophochloa pheloides*) to n=36 (*Digitaria adscendens*). Data on basic chromosome numbers* for different genera and the recorded haploid chromosome numbers (cf. table 1) reveal the existence of a great amount of chromosomal variability in grasses as reflected by the high frequency of eu- and aneu-ploid series.

High frequency of polyploids in grasses is also supported by the present studies. Amongst the worked out taxa as many as 64.6% were polyploids, which pertain to mostly the tetraploids (50%) and hexaploids (45%). Roughly speaking, the incidence of ploypoidy amongst grasses is almost twice the average frequency of polyploids in the flowering plants (see Stebbins 1956, Mehra 1982). Presently, by analysing the habit (see foot note* to table 1) and the chromosome numbers of the worked out taxa (table 8), it has been observed that the percentage of polyploid species amongst perennial grasses was higher (85.7%) as compared

*According to Mehra (1982), all the major grass taxa have evolved from a Programineous stock with base number 6 which ultimately gave rise to other numbers as n=7, 9, 10, 11 and 12 as represented in various tribes and genera

Table 7 *Morphological characteristic of diploid and tetraploid cytotypes in* Dactyloctenium aegyptium

S. No.	Characters	Diploid n=9	Diploid n=10	Tetraploid n=19
1.	Stem: Height	29–40 cm	24–32 cm	23–28 cm
2.	Leaf*			
	(*a*) Sheath length	5.0–5.5 cm	4.5–6.0 cm	4.8–5.7 cm
	(*b*) Lamina length	7–10 cm	6.5–8.0 cm	5–6 cm
	(*c*) Lamina breadth	2–3 mm	5–6 mm	5–6 mm
3.	Stomata**			
	(*a*) Length	34–42 μm	34–35 μm	42–50 μm
	(*b*) Breadth	11–15 μm	7–15 μm	7–11 μm
	(*c*) Frequency/unit area	4–5	3–4	3–4
4.	Inflorescence length	1.5–2.5 cm	3.0–3.5 cm	2.5–3.0 cm
5.	Pollen**			
	(*a*) Size (Length × Breadth)	28–31 × 25–27 μm	30–33 × 28–30 μm	32–35 × 29–3 μm
	(*b*) Fertility	99.80%	100.00%	100.00%

*Based on 10 samples
**Based on 50 samples

Table 8 *Diploid-Polyploidy correlation in the investigated grass species/taxa*

Species	Total	Diploid*	Polyploid*	%Polyploidy
Perennial	7/13	2/3	6/10	85.7/76.9
Annual	15/18	7/8	10/10	66.6/55.5
Total	22/31	9/11	16/20	72.7/64.5

*The difference in the number is due to the fact that three species with different cytotypes (*Dactyloctenium aegyptium*, 2x, 4x; *Lophochloa pheloides*, 2x, 4x and *Saccharum bengalense*, 2x, 6x) have been counted on the diploid and polyploid sides

to the annual grasses (66.6%). Almost same conclusion holds good if analysis is based on taxa basis. Interestingly, Sharma and Khosla (1981) concluded that the incidence of polyploidy in the Himalayas decreases with increasing latitudes in change from sub-tropical to sub-temperate and temperate climates.

Quite a many species have been found to show intraspecific polyploid races. The cumulative data on the cytology of presently studied species of grasses as provided in table 1 show the existence of intraspecific polyploid series in as many as 72.7% analysed species. In *Saccharum bengalense*, the diploid (n=10), tetraploid (n=20) and

hexaploid (n=30) cytotypes can be identified (cf. table 3). The hexaploids are well stabilized and are very common. As compared to the characters mentioned by Mehra et al. (1968) the various cytotypes show some variability in the size of the plant, the thickness of the culm and the length of inflorescence. However, these show a real constancy in the characters of palea and leaf sheath colours. Obviously, the minor morphological differences in various cytotypes could be attributed to some variability in weather and growth conditions in the region from year to year. In case of *Saccharum spontaneum* also, the four cytotypes (n=20, 27, 28, 29) recorded by the authors, are separable on the basis of characters of inflorescence and leaves (cf. table 5). Amongst these, only two populations were tetraploid and from within the populations exhibiting aneuploid numbers at hexaploid level (n=27, 28, 29), n=27 was more commonly found. Diploid population of *S. bengalense* with n=11 and hexaploid populations of *S. spontaneum* with n=27, 28, 29 clearly bring out the role of aneuploidy in the evolution of new biotypes. In case of both *S. bengalense* and *S. spontaneum* the role of a new genotype is supported to a lesser or greater extent by identifiable phenotypes.

Mehra and Kalia (1973) have reported the existence of four cytotypes A (n=10), B (n=10+1′,11), C (n=11+1′, 12) and D (n=12+1′) of *S. bengalense*. These invariably have 2n=20 in the somatic cells. They referred the extra chromosomes as univalent accessories or B-chromosomes. Amongst the diploid of this grass we observed only 10_{II} and 11_{II} and in none of PMCs with clearly countable 10_{II} or 11_{II} any extra chromosome or chromatin body was found. Here it is interesting to point out that in case of *Sesbania bispinosa* (Bir et al. 1975, Bir & Sidhu 1981) plants with 2n=12, 13 (trisomic) and 14 (tetrasomic) do exist in nature. In case of *S. bengalense* population with n=11 there is always 11+11 distribution at A-I and never the extra two chromosomes (over n=10) behave as accessory or B-chromosomes. There is thus a possibility of additional homologous chromosomes in *S. bengalense* cytotypes with n=11, 12, 13 (present investigation as well those of Mehra & Kalia, loc. cit.), the original diploid number is n=10.

Presently, the phenomenon of cytomixis has been noted to operate at various stages of meiosis (from prophase to telophase) in cytotypes γ & δ *Saccharum bengalense* with n=20 and n=30 respectively. This causes numerical variation in chromosomes and generally the gametes with variable chromosome numbers (both hypo- and hyperploid) are the result but as compared to normal ones those with less than the normal haploid complement of chromosomes, may not be so competitive in fertilization. Ultimately, this accounts for the great variation in the chromosome numbers in grasses. The variable cytotypes thus coming into existence are perpetuated through vegetative propagation so common in the group. Multinucleate pollen mother cells with variable number of chromosomes have presently been observed in hexaploid cytotype-δ (normally with n=29) of *S. spontaneum*. Earlier, such cells are reported for *S. robustum* (Price 1956) and *S. bengalense* (Mehra & Kalia 1973). One of such PMC showed 16_{II}, 28_{II}, 52_{II} and 150_{II}. According to Price (1956) these multinucleate PMCs are the result of (*i*) failure of cytokinesis in the mitotic division of the archesporial cells prior to meiosis, (*ii*) passage of nuclear material from one PMC to another and (*iii*) the fusion of PMCs. According to Mehra and Kalia (1973) the formation of multinucleate PMCs in *S. bengalense* is due to the fusion of PMCs which have very feeble cell walls. They have observed the different

nuclei to have 20 or multiples of 20 chromosomes. In the present case, since the different nuclei in the giant PMC have different chromosome numbers there is a great possibility of their formation due to irregular pre-meiotic division and consequential irregular cytokinesis in archesporial cells. About the evolutionary significance of such multinucleate cells, it has often been suggested (Price 1956) that these lead to the production of polyploid taxa, but the formation of polyploid viable gametes has generally been doubted. At the same time the possibility of involvement of the genetically variable pollen from multinucleate PMCs in the origin of aneuploid cytotypes can not be ruled out. It seems that the phenomenon of cytomixis as well as the incidence of multinucleate PMCs have contributed to the origin of various chromosome numbers in case of *S. bengalense* and *S. spontaneum* in particular and grasses in general.

Summing up, it is clear that polyploidy and aneuploidy have played an important role in the evolution of new taxa in grasses. The phenomenon of cytomixis and the incidence of multinucleate PMCs add to the genetical variability. Apomictic mode of reproduction (Stebbins 1956) and the extensive vegetative propagation in grasses are helpful in the stabilization of the new genotypes in nature. This is the reason we come across more frequently genetically variable populations in grasses as compared to the dicotyledons plants where the newly evolved genotypes are not stabilized easily and are eliminated.

Acknowledgements

One of us (MS) is thankful to CSIR New Delhi for the award of research fellowship during the course of present investigations.

References

Bir S S and Sidhu M 1981 Cytological studies on weeds–I *Sesbania bispinosa; Curr. Sci.* **50** 281–282

——, ——and Talwar K 1975 Cytological observations on genus *Sesbania*f rom the Punjab Plain (North India); *New Botanist* **11** 101–108

Bor N L 1960 *The Grasses of Burma, Ceylon, India and Pakistan* (Pergamon Press)

Darlington C D and Wylie A P 1955 *Chromosome Atlas of Flowering Plants* (London: George Allen Unwin Ltd.)

Fedorov An A (ed.) 1969 *Chromosome Number of Flowering Plants* (USSR: Komarov Botanical Institute) 926 pp

Love A and Love D 1961 *Chromosome Numbers of Central and North Western European Plant Species* (Stockholm: Opera Botanica Alvquist & Wikselli)

—— and —— 1974 *Cytotaxonomical Atlas of the Slovanian Flora* (Lehre: J. Crammer Fl.)

—— and —— 1975 *Cytotaxonomical Atlas of the Arctic Flora* (Lehre: J. Crammer Fl.) 3301

Mehra P N 1982 *Cytology of East Indian Grasses* (New Delhi)

—— and Kalia Vipan 1973 Accessory chromosomes and multinucleate pollen mother cells in *Saccharum bengalense* Retz.; *Complex Nucleus* **XVI** (ii) 75–88

——, Khosla P K, Kholi B L and Koonar J S 1968 Cytological studies in the North Indian grasses I; *Res. Bull. Panjab Univ.* (*n.s.*) **19** (1) 157–230

—— and Sunder Shyam 1970 Cytological studies in the North Indian grasses II; *Res. Bull. Panjab Univ.* (*n.s.*) **20** 503–539

Price S 1956 Cytological studies in *Saccharum* and allied genera I. Snycytes in certain clones of *Saccharum* and *Erianthus; Cytologia* **21** 21–37

Sharma M L and Khosla P K 1981 Polyploidy in the Himalayan grasses; in *Contemporary Trends in Plants Sciences* ed. S C Verma (New Delhi: Kalyani Publishers) 432 pp

—— and Parveen Kumar 1980 In IOPB Chromosome number reports LXIV; *Taxon* **29** (5:6) 703–730

Stebbins G L 1956 Cytogenetics and evolution of grass family; *Amer. Jour. Bot.* **43** 890–905

Proc. Indian natn. Sci. Acad. B51 No 5 pp 627–632 (1985)

Grain Yield and Yield Components of Regenerated Wheat Plants in SC-4 Generation

SUSAN EAPEN[1], K N SUSEELAN[2], S G BHAGWAT[2], P S RAO[1] and C R BHATIA[2]
Bio-Organic Division[1] and Nuclear Agriculture Division[2],
Bhabha Atomic Research Centre, Trombay, Bombay 400085

(Received 16 July 1985; after revision 18 November 1985)

Plants were regenerated from callus cultures initiated from mature embryo explants of two bread wheat cultivars Kalyan Sona and NI-4. The regenerated (SC-1) plants of cultivar NI-4 showed chlorophyll variants such as albino and striata. No such variant was observed in cv. Kalyan Sona. Progenies of NI-4 segregated for chlorophyll variants up to SC-3 generation. The highest yielding SC-3 plants derived from each of the original SC-1 plants were selected and their progenies evaluated for grain yield and yield components in replicated field experiments. All the 18 regenerated lines of Kalyan Sona were statistically similar to the control in grain yield. Most of the yield components, gliadins and high molecular weight glutenins were comparable to the parent. In cultivar NI-4, which showed greater phenotypic variability, 6 out of 12 regenerated lines were equal in grain yield. The results indicate that some of the unwanted genetic alterations induced by *in vitro* culture are partly eliminated by subsequent cycles of sexual reproduction and can be further reduced by conventional plant breeding methods of selecting parental and higher yielding single plants to recover lines equal in productivity to the original cultivar.

Key Words: **Biological yield, Chlorophyll variants, Gliadin, Glutenin, Grain yield**

Introduction

Heritable somaclonal variation is known to occur in tissue cultures of cereals like rice (Oono 1978, Fukui 1983, Sun et al. 1983) and wheat (Larkin et al. 1984). When techniques of genetic manipulations are applied at the cellular level for specific characters, it is essential that the modified plant is comparable in productivity with the parent material. Larkin et al. (1984) have made extensive studies of many morphological and biochemical traits in somaclones of wheat. However, little attention has been focussed on the yield and various components of yield of wheat somaclones. The present study was undertaken to evaluate variation in grain yield and yield components in somaclonal—SC-4 generation of bread wheat. In addition, storage proteins—gliadins and high molecular weight glutenin

subunits of SC-4 lines of one cultivar were investigated by electrophoresis on acid and SDS gels respectively to assess the extent of alterations for storage proteins.

Materials and Methods

Bread wheat (*Triticum aestivum* L. Thell) cvs Kalyan Sona and NI-4 were used in the study. Callus cultures were initiated from mature embryos obtained from seeds as previously described (Eapen & Rao 1982). Callus cultures were initiated from 200 embryos of Kalyan Sona and 100 of NI-4 on medium containing mineral elements of Murashige and Skoog (Murashige & Skoog 1962) supplemented with Lin and Staba's vitamins (Lin & Staba 1961), 2,4,5-trichlorophenoxy acetic acid (5 mg/l) and 2% sucrose. At the end of second passage the cultures were transferred to basal medium supplemented with zeatin (1 mg/l) and indoleacetic acid (0.1 mg/l). The regenerated plants (SC-1 generation) were transferred to paper cups and then to pots in the field. Seeds obtained from 19 Kalyan Sona and 12 NI-4 SC-1 plants were used for raising the SC-2 generation in the pots. SC-3 population was also grown in pots and harvested as single plants. Among the plants derived from original regenerant, plants which showed maximum grain weight were selected for evaluation in the SC-4 generation. The SC-4 plants were grown in the field as plant to row progenies with four replicates. Each row was 3 m long. The distance between rows was 30 cm and seeds in a row were planted with a spacing of 7 cm. Normal irrigation and cultural practices were followed. Urea was applied in two instalments as top dressing at tillering and boot-leaf stage at the rate of 60 kg/ha each.

Plant height was measured before harvest from ground level to the tip of the ear, excluding awns. At harvest, plants in each row were pulled out, counted and weighed to estimate biological yield (BY). Grain yield (GY) was estimated after threshing. Yield components like number of effective tillers, number of spikelets per ear, number of seeds per ear were estimated from 5 plants per row in each replication. Other characters studied include thousand grain weight, days for heading, glume colour, grain colour and speltoid mutations.

Electrophoresis

Seeds obtained from 18 SC-4 lines of Kalyan Sona control were ground in a Udy cyclone mill. Whole meal was used for electrophoresis following the procedures described by Bushuk and Zillman (1978) for gliadins and Payne et al. (1980) for high molecular weight glutenins.

Results

In cv-NI-4, shoot buds were produced in 50% of the cultures, whereas in Kalyan Sona, only about 10% of the cultures differentiated shoot buds. Chlorophyll variant including albinos and striatas were observed in about 10% cultures of NI-4 whereas in Kalyan Sona no chlorophyll variant was observed. None of the chlorophyll variants survived up to the seed-setting stage. Many plants were sterile and seeds could be collected only from 19 SC-1 plants of Kalyan Sona and 12 of NI-4 out of 65 and 40 regenerants transferred to pots (table 1). These were used for raising the SC-2 generation.

The SC-2 and SC-3 plants were comparable to the normal seed grown plants in morphological and physiological characters. However, in NI-4, two albino plants were observed in SC-2 and one in SC-3. The frequency distribution of grain weight of SC-3 plants of Kalyan Sona and NI-4 are given in table 2. Among Kalyan Sona SC-3 plants, 18 regenerants were selected having grain weight above 11.6 g and in NI-4 12 lines were selected having grain weight above 11.3 g.

Table 1 *Number of wheat plants regenerated and evaluated in different somaclonal generations*

Generation	Number of plants/plant progenies	
	cv. Kalyan Sona	cv. NI-4
SC-1		
Regenerated plants transferred to pots	65	40
Plants survived to produce seeds	19	12
% Survival	29.2	30
SC-2	96	31
SC-3	384	116
SC-4 plant progenies selected from SC-3	13	12

Table 2 *Frequency distribution of grain weight of SC-3 regenerants of wheat*

Grain weight (g)	Frequency Distribution	
	NI-4	Kalyan Sona
0–5	51	226
5–10	49	121
10–15	15	27
15–20	3	9
20–25	Nil	1

Variation in SC-4 Generation

Kalyan Sona: Control and all other lines except KS-9, KS-13 and KS-18 came to heading 45 days after sowing, the other lines were late by 5 days. Differences in grain yield. biological yield, number of tillers and thousand grain weight were not significant (table 3). The number of spikelets/ear and number of seeds/ear were significantly higher than control in 2 and 1 lines. Five lines of Kalyan Sona were shorter and one significantly taller than the parent cultivar. Five lines segregated for speltoid mutants and their frequencies ranged from 0.5 and 2.0%. No such mutant was observed in control and other lines.

NI-4: No chlorophyll variant was observed in SC-4 population of NI-4. Control and other lines flowered in 60 days except NI-4-1 which was earlier by 10 days. SC-4 lines of NI-4 showed significant variability for grain yield and grains/ear 3, 11 and 10 lines were significantly lower for these parameters. One line with significantly lower number of seeds/ear had higher 1000 grain weight (table 4). One line of NI-4 was significantly shorter than control and other lines.

Electrophoresis of Proteins

No detectable differences were observed in the gliadin or high-molecular-weight glutenin patterns of the regenerated plants of Kalyan Sona. The 18 regenerated lines analysed showed protein patterns identical to the control.

Discussion

The variation observed in the regenerated plants and their progenies in the present experiments is similar to that reported by Larkin et al. (1984). They found heritable variation in plant height, awns, tiller number, grain colour, heading date, waxiness, glume colour, gliadin proteins and α-amylase regulation. Since the first comprehensive studies of Mac Key (1954), phenotypically similar mutants affecting the above mentioned traits have been repeatedly observed in mutation experiments of wheat following radiation and chemical mutagen treatments.

In the present study, chlorophyll mutations were observed up to SC-3 generation of NI-4. Such mutants are not observed in the regenerated plants of Kalyan Sona or their progenies.

Table 3 *Yield and yield components SC 4 plants of breadwheat cv. Kalyan Sona*

SC 4-line	GY g/m²	BY g/m²	No. of tillers	Height cms	No. of spikelets per ear	No. of seeds per ear	1000 grain weight
KS-C	257	875	4.8	78.6	14.8	38.2	30.9
KS-1	246	913	4.9	77.6	15.3	42.1	28.6
KS-2	203	763	4.4	77.0	14.8	37.5	28.3
KS-3	197	750	4.8	76.6	14.7	44.2	27.7
KS-4	240	900	4.4	80.4	15.2	43.5	28.5
KS-5	241	900	4.5	78.2	15.4	41.9	27.9
KS-6	225	825	5.4	77.8	15.6	45.5	27.7
KS-7	240	900	4.6	80.0	15.2	42.8	29.2
KS-8	241	900	4.9	79.6	15.3	41.3	27.9
KS-9	249	950	5.2	78.4	15.2	44.9	28.0
KS-10	223	913	6.5	75.8	14.7	42.5	25.2
KS-11	228	925	5.6	69.2	15.6	50.0*	27.2
KS-12	241	925	5.6	72.8	16.2*	44.0	27.3
KS-13	195	825	4.8	93.0*	15.9	35.0	31.1
KS-14	242	888	5.3	67.6	15.7	43.0	27.2
KS-15	205	888	4.7	71.0	16.6*	44.1	28.0
KS-16	176	863	5.2	71.2	15.7	46.8	27.5
KS-17	224	900	5.0	72.2	15.4	39.6	27.0
KS-18	225	888	6.3	72.2	15.8	46.8	26.4
LSD-5%	NS	NS	NS	4.6	0.8	6.9	NS
LSD-1%	NS	NS	NS	5.5	1.1	9.0	NS

* Significantly higher than control

NS, Not significant; *GY*, Grain yield; *BY*, Biological yield

Table 4 *Yield and yield components of SC 4 plants of breadwheat cv. NI-4*

SC 4-line	GY g/m²	BY g/m²	No. of tillers	Height cms	No. of spikelets per ear	No. of seeds per spike	1000 grain weight
NI-4-C	287	1325	6.3	102.0	15.0	32.5	39.5
NI-4-1	195	900	5.3	94.8	13.9	23.4	42.6*
NI-4-2	234	1200	4.6	105.6	14.9	31.1	39.8
NI-4-3	217	1225	4.6	107.8	13.5	27.9	40.7
NI-4-4	176	1025	5.3	100.2	14.3	23.4	41.4
NI-4-5	211	1213	5.2	105.2	13.7	22.9	41.8
NI-4-6	243	1188	6.4	106.8	14.1	26.4	42.3
NI-4-7	241	1200	5.3	110.0	15.0	27.6	41.2
NI-4-8	210	1025	5.2	113.6	14.4	23.6	42.7
NI-4-9	230	1225	6.0	111.6	14.0	24.6	38.0
NI-4-10	224	1075	7.2	111.2	14.9	26.3	38.0
NI-4-11	195	967	6.1	112.6	14.4	26.2	39.0
NI-4-12	253	1325	5.2	103.2	14.2	31.4	40.8
LSD-5%	51	NS	NS	8.5	NS	1.7	2.2
LSD-1%	68	NS	NS	11.3	NS	2.2	3.0

* Significantly higher than control

NS, Not significant; *GY*, Grain yield; *BY*, Biological yield

None of the 21 nullisomics of the cultivar Chinese Spring show lack of chlorophyll (Sears 1972) indicating that the genes for chlorophyll development are located on more than one chromosome. Unless two or more loci are affected simultaneously, mutation relating to chlorophyll development cannot find expression. Chlorophyll mutation following mutagenic treatment are either absent or extremely rare in most wheat cultivars (Bhatia & Swaminathan 1963, Sears 1972). Among the several Indian cultivars used in mutation experiments, only one cultivar C-591 repeatedly produced chlorophyll mutations in high frequencies (Natarajan et al. 1958, Bhatia & Swaminathan 1963) suggesting that alterations at a single locus may lead to chlorophyll mutations in this cultivar. Subsequently this was found to be so when nulli 3A of C-591 were found to lack chlorophyll development (Singh & Joshi 1979). Thus in some wheat cultivars a single locus deletion or mutation leads to lack of chlorophyll production. The high frequency of chlorophyll deficient segregants in NI-4 suggests that this cultivar may also have non-duplicated locus for chlorophyll development. Segregation of albinos in SC-2 and SC-3 generation is also reported in rice somaclones (Sun et al. 1984).

In bread wheat, spontaneous speltoid mutants are quite frequent. Mac Key (1954) made a detailed study of spontaneous and radiation induced speltoid mutants. The spontaneous frequency was between 0.6–0.7% in wheat cultivars used in his experiments. Mutation frequencies ranging from 0.5–2.0% were observed in five SC-4 progenies of Kalyan Sona. No speltoid mutant was observed in equivalent control population though speltoids do occur spontaneously in this cultivar.

In the present study we have analysed the grain yield and yield components of regenerated SC-4 lines of wheat. For possible utilization of the *in vitro* techniques in wheat breeding programmes, it would be necessary to avoid unwanted genetic variation. One approach could be to develop better *in vitro* culture methods. In the present study, most of the unwanted genetic variation was eliminated due to the low survival rate and sterility in SC-1 generation. Subsequent selection of SC-3 plants showing maximum grain weight helped in further elimination of undesirable genetic variation. Grain yield of all 18 regenerated lines of Kalyan Sona were statistically equal to the control. Most yield components were not significantly different in the regenerated lines of Kalyan Sona. Some of the Kalyan Sona regenerated lines showed positive improvements in the desired direction like increased number of spikelets and grains per ear though selections were not made on these components. Such variations in quantitative characters are common in mutation experiments, but are not easily used for yield improvements. Gliadin and high molecular weight glutenin proteins were also identical to the original cultivar. The results indicate that the unwanted genetic variation induced by *in vitro* culture are partly eliminated in subsequent cycles of sexual reproduction and can be futher reduced by selection of parental and higher yielding types to recover lines equal in productivity to the original cultivars.

References

Bhatia C R and Swaminathan M S 1963 Frequency and spectrum of mutations induced by radiations in some varieties of bread wheat; *Euphytica* **12** 97–112

Bushuk W and Zillman R R 1978 Wheat cultivar identification by gliadin electropherograms. I. Apparatus, method and nomenclature; *Can. J. Pl. Sci.* **58** 505–515

Eapen S and Rao P S 1982 Organogenesis and plantlet formation from callus cultures of different cultivars of breadwheat (*Triticum aestivum*); *Proc. Indian natn. Sci. Acad.* **B48** 371–377

Fukui K 1983 Sequential occurrence of mutations in a growing rice callus; *Theor. Appl. Genet.* **65** 225–230

Larkin P J, Ryan S A, Brettel R I S and Scowcroft W R 1984 Heritable somaclonal variation in wheat; *Theor. Appl. Genet.* **67** 443–457

Lin M and Staba J 1961 Peppermint and spearmint tissue cultures. Callus formation and submerged culture; *Lloydia* **24** 139–145

Mac Key J 1954 Neutron and X-ray experiments in wheat and revision of the speltoid problem; *Hereditas* **40** 65–180

Natarajan A T, Sikka S M and Swaminathan M S 1958 Polyploidy, radiosensitivity and mutation frequency in wheats. Proc. 2nd UN Int. Conf. Peaceful Uses Atom. Energy; *Geneva* **27** 321–331

Oono K 1978 Test tube breeding of rice by tissue culture; *Trop. Agric. Res. Ser.* **11** 109–124

Payne P I, Law C N and Mudd E E 1980 Control of homologous group 1. chromosomes of the high molecular-weight subunits of glutenin, a major protein of wheat endosperm; *Theor. Appl. Genet.* **58** 113–120

Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassay with tobacco tissue culture; *Physiol. Plant.* **15** 473–497

Sears E R 1972 The nature of mutation in hexaploid wheat; *Symp. Biol. Hung.* **12** 73–82

Singh D and Joshi B C 1979 Chlorophyll synthetic gene(s) in *T. aestivum* var (pb C 591); *Wheat Inf. Ser.* **50** 45–46

Sun Z-x, Zhao C-z, Zheng K-l, Qi X-f and Fu Y-p 1983 Somaclonal genetics of rice; *Theor. Appl. Genet.* **67** 67–73

Proc. Indian natn. Sci. Acad. **B51** No. 5 pp 633-640 (1985)

Influence of Water Stress and Clipping on Growth Performance and Nutrient Value of *Chloris gayana* Kunth.

HEMA JOSHI
Department of Botany, Kumaun University, Nainital 263 002

(Received 24 April 1984; after revision 14 October 1985)

The influence of water stress and clipping treatments on growth behaviour and nutrient value of *Chloris gayana* was studied. Total biomass, biomass of shoots, net production and relative growth rates (except R_RGR of weekly clipped plants) declined due to water stress and clipping frequency. In contrast, root biomass and root : shoot ratio increased under water stress but were depressed due to clipping. Effect of water stress was not marked on unit leaf rate but the unclipped plants showed a higher rate compared to the clipped ones. Water stress and clipping caused a reduction in total non-structural carbohydrate content of different organs. Protein content in shoots declined due to water stress but was increased by clipping.

Key Words: **Water stress, Clipping, Growth behaviour Nutrient value, Chloris gayana kunth**

Introduction

The most frequent cause of water stress in plants is a suboptimal soil water supply coupled with the rate of transpiration being in excess of the rate of absorption of water by roots. Several researchers have demonstrated that under conditions of water stress, growth of plants is usually reduced. However, different plants respond differentially to water stress conditions. In addition to water stress, plant growth is also affected by herbage removal through grazing or clipping. Previous studies have shown that herbage yield, vigour and TNC (Total non-structural carbohydrate) levels of plants are drastically reduced by intensive defoliations (Trlica & Cook 1971, Bokhari & Singh 1974, Owensby et al. 1974).

The present study was undertaken to examine the influence of water stress and clipping frequency on growth behaviour and nutrient value of *Chloris gayana* Kunth. (Rhodes Grass).

Method of Study

Plant material

Tillers of *C. gayana* were obtained from the experimental farm of Indo-German Agricultural Development Agency (IGADA at Almora). The tillers of uniform size were transplanted into polyethylene pots filled with weighed amount of a mixture of soil and farmyard manure (3 : 1) and grown in a glass house at Nainital from July to

November, 1977. Temperature in the glass house ranged between 13°C (average minimum) and 25°C (average maximum). Nainital is located at 2050 m above mean sea level (29°24'N 79°28'E) and experiences a monsoon temperate climate (Pandey & Singh 1980).

Soil water conditions

Two levels of water stress were maintained as described by Pande and Singh (1981). In brief, for the first two weeks the pots were watered regularly to maintain the soil water at the level of maximum water holding capacity. After this period soil water content in one set of pots was maintained at full water holding capacity (1 WHC) while in the other set the soil was allowed to dry to a level as close as possible to half water holding capacity ($\frac{1}{2}$ WHC). Under both water conditions pots were watered every third or fourth day and soil water was brought to the desired level (i.e. 1 WHC or $\frac{1}{2}$ WHC) by adding the required amount of water. In addition to the regular weighing, soil water content was monitored gravimetrically at frequent intervals 72 pots (one plant per pot) were maintained under 1 WHC and 72 under $\frac{1}{2}$ WHC. Variations in gravimetric water content within treatments were little as reported in Pande and Singh (1981).

Clipping treatment

Plants were divided into four sets (18 pots per set) under each water condition. One set of 18 pots was treated as control (unclipped). Out of the remaining three sets, one set each was subjected to weekly, fortnightly and monthly clipping treatments. First clipping was identified as time zero. The height of clipping was fixed so as to remove 80% shoot, by volume, from each plant. Thus, the clipping height was fixed on the first sampling date and on subsequent samplings the same height was maintained. Clipping height from the base of the tiller was 9.6 cm. The clipped material was oven dried and weighed on each treatment date.

Biomass

The biomass was evaluated at fortnightly interval by oven-drying at 80°C. In this paper shoot weight was the sum of cumulative weights of leaf and stem plus the weight of crown. The weight of the material clipped between the sampling dates S_1 and S_2 was added to the shoot weight for the sampling date S_2.

Growth parameters

Various growth parameters such as total leaf area (TLA), specific leaf area (SLA), relative growth rate (RGR), unit leaf rate (ULR), leaf weight ratio (LWR) and leaf area ratio (LAR) were calculated following Evans (1972).

Chemical analysis

Total non-structural carbohydrates (TNC) of shoots, roots and crowns were determined following Smith (1969). The nitrogen content was determined for shoots only, through the micro-Kjeldhal procedure as given by Piper (1944). Protein content was then calculated by multiplying N content by 6.25. Chemical analysis of plant material was done at I, IV and VI harvests. Each analysis was replicated thrice. For chemical analysis shoot refers to leaves + stem.

Results and Discussion

Plant biomass

Cumulative total biomass as well as biomass of different organs tended to increase with time attaining the highest values at final harvest (figure 1). The biomass varied significantly with water level, clipping frequency (shoot biomass $P<0.005$; root and total biomass $P<0.001$) and sampling date

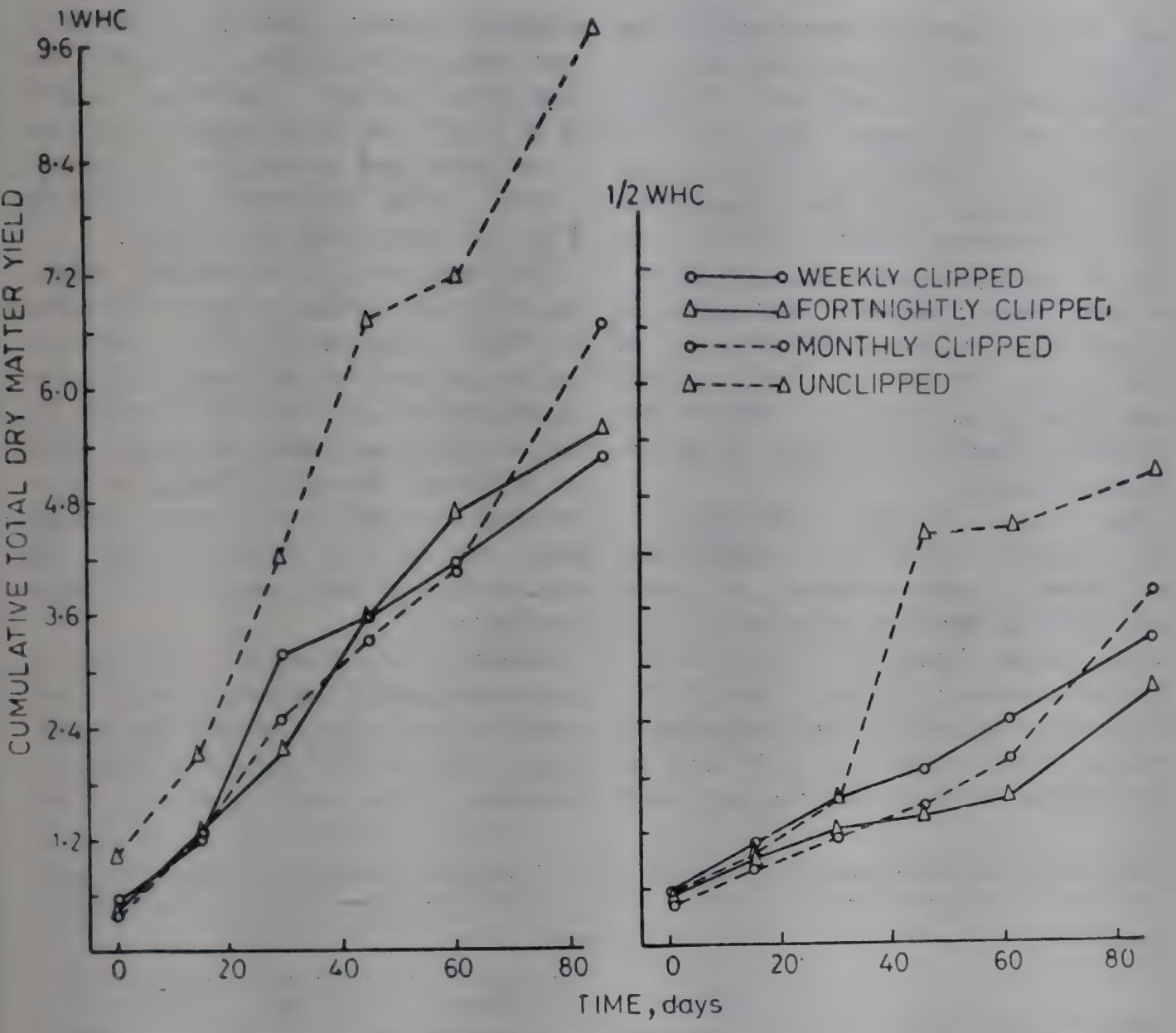


Figure 1 Cumulative total dry matter yield of *Chloris gayana* subjected to various clipping treatments under two soil water conditions

(all $P<0.001$). Interactions between sampling date × clipping frequency (in all $P<0.001$) water level × sampling date (in total biomass $P<0.05$) and water level × clipping frequency (in total biomass $P<0.05$) were also significant.

Water stress reduced cumulative total biomass and shoot biomass under all clipping treatments. Reduction in biomass due to water stress was greatest for weekly clipped plants (55% reduction in total biomass and 50% in shoot biomass) followed by unclipped (total biomass 39% and shoot biomass 45%) fortnightly clipped (total biomass 35% shoot biomass 42%) and monthly clipped plants (total biomass 80% and shoot biomass 11%). In contrast to shoot and total biomass, root biomass was increased under water stress (table 1). This increase in root biomass was in the order : fortnightly clipped (93%)>weekly clipped (38%)> monthly clipped (16%)>unclipped plants (7%). Increase in root biomass may be due to some stimulation of root growth by slight moisture stress. This has also been reported for certain other species (Jarvis 1963). Under

both water conditions increasing clipping frequency adversely affected total biomass and biomass of shoots and roots. Clipping affected the root system more adversely compared to shoot system. It was suggested (Bokhari & Singh 1974) that frequent clipping and consequent recovery allows little time for the manufacture of surplus photosynthate, hence, downward translocation is limited. This results in the reduction of root growth. Painter and Detling (1981) and Detling et al. (1979) also observed that increased frequency of clipping of grasses reduces root yield.

Root: Shoot ratio: Under both water conditions, the (R : S) ratio of unclipped plants was generally higher compared to clipped ones, although the time series pattern was rather irregular (figure 2). Under 1 WHC, with few exceptions there was a gradual decline in R : S ratio with time in all clipping treatments while under 1/2 WHC after an initial decline the values stablished or showed temporary increase. The R : S ratio values were comparatively higher in plants under water stress condition. According to Loomis et al. (1971), the water stress slows shoot growth more and sooner than it does root growth. Under both water conditions the R:S ratio declined due to clipping.

Net Production: It is evident from table 2 that total net production was lower under 1/2 WHC compared to 1 WHC. Net production in roots of all clipped plants increased due to water stress while that of unclipped plants was slightly depressed. Total net production and net production in shoots exhibited least reduction in monthly clipped plants but in other three treatments the reduction due to water stress was of a similar magnitude.

Generally, net production decreased by increasing clipping frequency (table 2). Compared to the unclipped plants, the clipped plants under 1 WHC exhibited lower net production. The root production was

Table 1 *Total root dry matter yield of* Chloris gayana (*at first & last harvest*) *under different treatments* (*g plant*$^{-1}$)

Plants	1 WHC		$\frac{1}{2}$ WHC	
	I Harvest	VI Harvest	I Harvest	VI Harvest
Weekly clipped	0.06±0.01	0.19±0.01	0.07±0.009	0.25±0.029
Fortnightly clipped	0.04±0.006	0.15±0.01	0.12±0.013	0.29±0.032
Monthly clipped	0.06±0.01	0.32±0.05	0.09±0.012	0.37±0.049
Unclipped	0.05±0.003	0.87±0.04	0.14±0.021	0.94±0.065

Table 2 *Net production of* Chloris gayana *during the experimental period under different treatments* (*g plant*$^{-1}$)

Plants	1 WHC			1/2 WHC		
	Shoot	Root	Total	Shoot	Root	Total
Weekly clipped	2.49	0.11	2.60	1.17	0.18	1.35
Fortnightly clipped	2.23	0.11	2.34	1.08	0.17	1.25
Monthly clipped	2.16	0.26	2.42	1.78	0.28	2.06
Unclipped	5.85	0.84	6.69	2.65	0.80	3.45

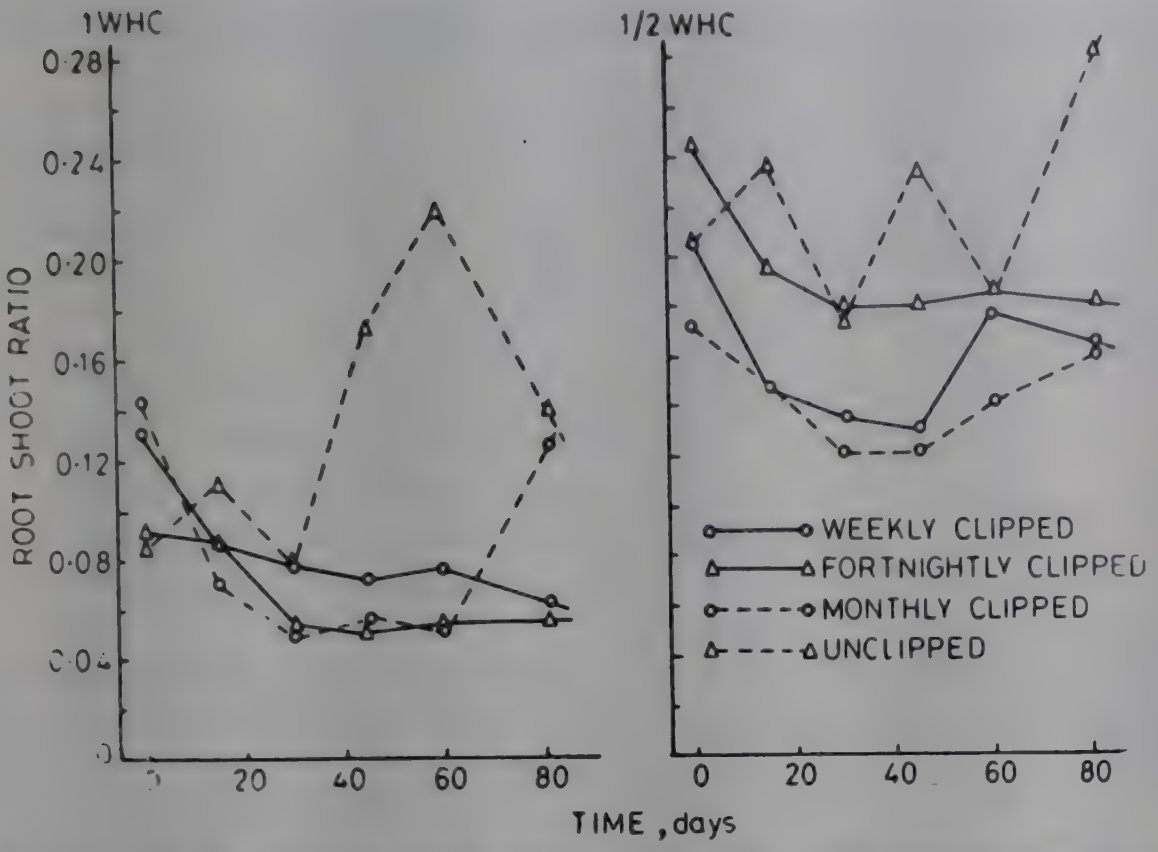


Figure 2 Root : shoot ratio of *Chloris gayana* subjected to various clipping treatments under two soil water conditions

comparatively more adversely affected by clipping compared to the shoot production. Again, generally, the clipping frequency affected net production to the same degree under both water conditions. For example, total net production of weekly and fortnightly clipped plants under 1 WHC was reduced by 62% and 65% and under 1/2 WHC by 61% and 64%, respectively.

Relative Growth Rate (*RGR*): Under both water conditions the RGR exhibited a declining trend (with few exceptions) in each treatment. The effect of species and treatments (water level × clipping) was significant at $p<0.05$ (figure 3). Declining RGR was also observed by Bokhari and Singh (1974) in *Agropyron smithi* and Higgs and James (1969) in *Agrostis* and *Lolium*.

The mean values of RGR indicated that water stress affected the R_wGR (RGR of whole plant) and R_sGR (RGR of shoot) adversely (table 3). Gates and Bonner (1959)

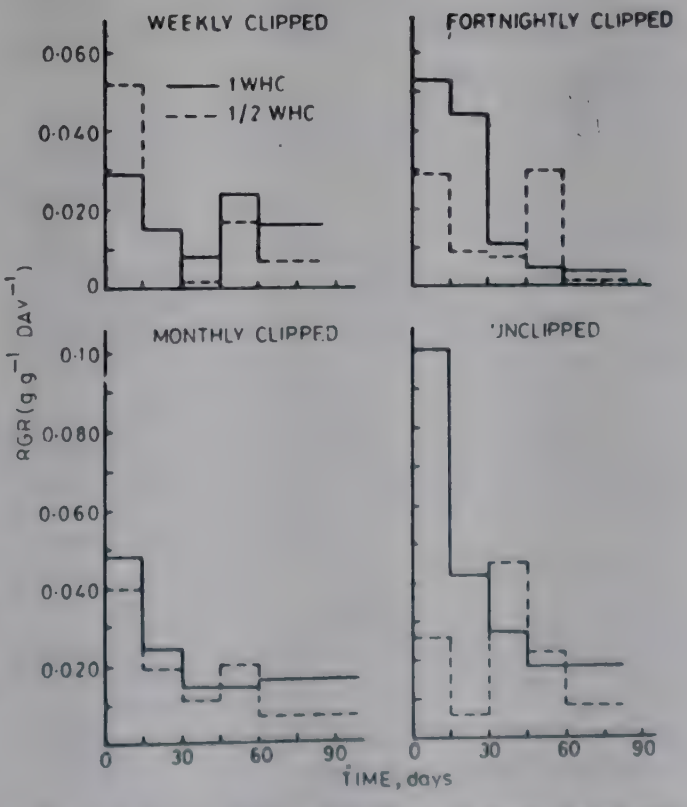


Figure 3 Relative growth rate of whole plant of *Chloris gayana* subjected to various clipping treatments under two soil water conditions

Table 3 *RGR (gg^{-1} day^{-1} and ULR (NAR) ($g\ cm^{-2} \times 10^{-5}$ day^{-1}) of* Chloris gayana *as mean across all sampling intervals*

Growth rate	1 WHC				½ WHC			
	Weekly clipped	Fort-nightly clipped	Monthly clipped	Un-clipped	Weekly clipped	Fort-nightly	Monthly clipped	Un-clipped
R_WGR	0.018	0.023	0.023	0.041	0.019	0.015	0.019	0.021
R_SGR	0.021	0.024	0.024	0.041	0.019	0.016	0.020	0.021
R_RGR	0.011	0.017	0.018	0.049	0.017	0.012	0.018	0.024
ULR	0.06	0.06	0.05	0.16	0.05	0.05	0.04	0.11

also observed that RGR of tomato was severely affected by drought; so much so that the RGR in water stressed plants dropped to zero. In the present study the R_RGR (RGR of root) of weekly clipped plants was stimulated by water stress. In other treatments it was reduced. Increasing clipping frequency also reduced the mean R_WGR (particularly under 1 WHC), R_SGR and R_RGR in all cases.

In general, the R_SGR: R GR ratio indicated that clipping shifted the balance between shoot and root growth so as to favour the former more strongly. This is in confirmation with the findings of other workers such as Kleinendorst and Brouwer (1969) and Davies (1974).

Unit Leaf Rate (ULR): Under both soil water conditions, in all treatments (with the exception of unclipped plants under (1/2 WHC) the trend in the rate of assimilation per unit leaf area generally appeared to be that of a decline. Mean values (table 2) showed that water stress did not affect the ULR but under both water conditions unclipped plants revealed a considerable greater rate than the clipped plants.

Total non-structural Carbohydrate (TNC): The unclipped plants indicated a continued increase in the TNC content during the experimental period showing that more of the reserves accumulates as the plants mature (figure 4). According to Mooney (1972), at the end of the growing season, as much as 60% dry matter in underground storage organs such as rhizomes may consist of carbohydrates. The clipping treatments interfered with this general pattern. Under 1 WHC root, shoot and crowns of clipped plants exhibited an initial increase in the TNC content followed by a decline. Similar trend was observed in *Artimisia tridentata* by Coyne and Cook (1970), under ½ WHC TNC content in all organs tended to decline rapidly with time.

Water stress adversely affected the TNC content of different organs. This decline was more marked in severely clipped plants than in moderately clipped plants. Trlica and Cook (1971) also reported a decline in carbohydrate reserves of sagebrush due to drought.

Clipping also caused a reduction in the TNC level (figure 3). The reduction was greater in severely clipped plants compared to moderately clipped plants. Again, clipping caused more reduction in the TNC levels of water stressed plants. The level of reduction due to severe clipping was greater for crowns and roots than for shoots.

Evidently the amount of assimilates produced by the remaining leaf area on severely clipped plants may be sufficient only for stimulated shoot growth. This will leave very little to be translocated to crowns and roots (Bokhari & Singh 1974). Willard and McKell (1978) and Trlica and Singh (1979) also observed low carbohydrate reserves in clipped or defoliated plants.

Crude Protein: Protein accumulation in shoots under two soil water conditions and various clipping treatments is depicted in figure 4. Water stress also had a depressing effect on protein content. According to Bonner (1950) water stress induces proteolysis in leaves. In contrast, increasing clipping frequency increased the protein content in shoots. Further, the water stressed plants subjected to serve clipping accumulated more protein compared to water stressed unclipped plants. The behaviour of protein content was thus reverse of TNC content. Besides increasing the protein content, clipping also resulted in greater relative leaf weight in mature plants.

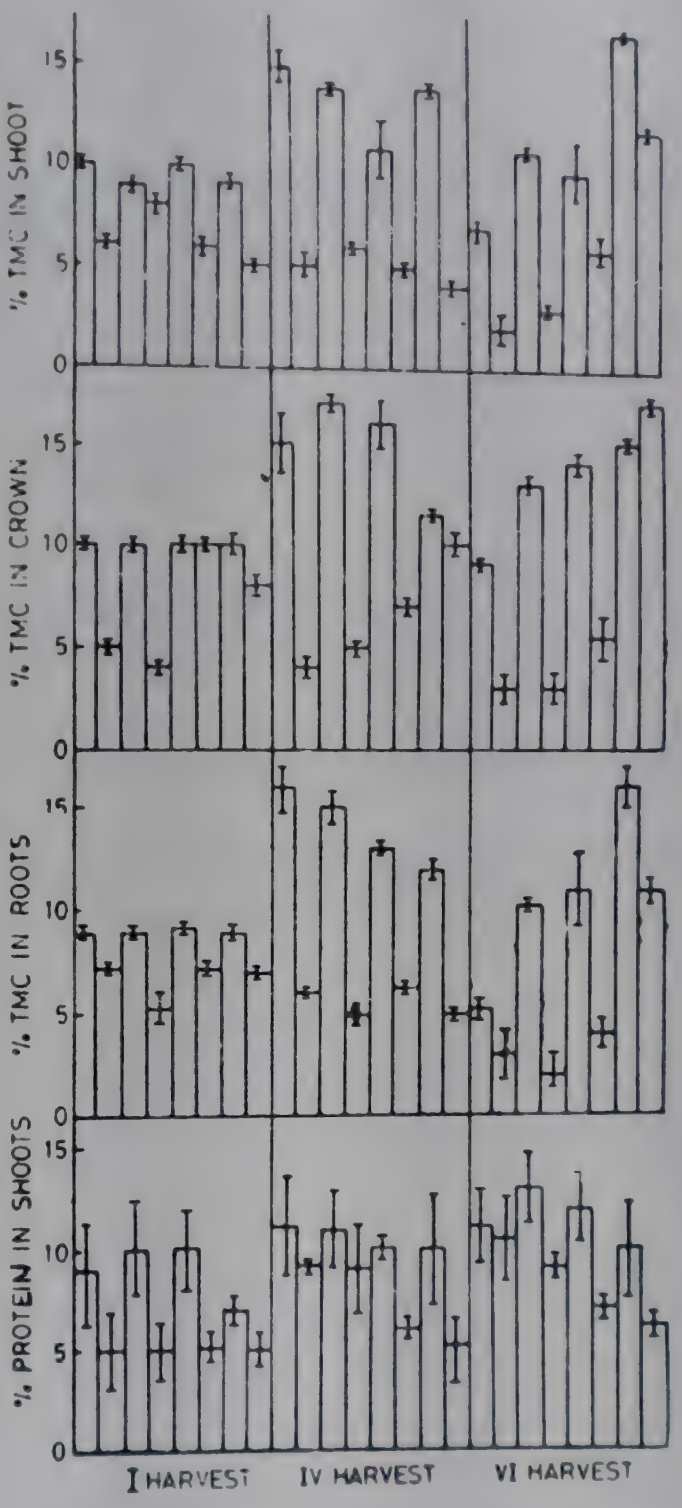


Figure 4 Total non-structural carbohydrate content of different organs and protein content of shoots of *Chloris gayana* subjected to clipping treatments under two soil water conditions. The bars from left to right represent: weekly clipped under 1 WHC, weekly clipped under ½ WHC, fortnightly clipped under 1 WHC, fortnightly clipped under ½ WHC, monthly clipped under 1 WHC, monthly clipped under ½ WHC, unclipped under 1 WHC and monthly clipped under ½ WHC →

References

Bokhari U G and Singh J S 1974 Effect of temperature and clipping on growth, carbohydrate, reserves and root exudation of western wheat grass in hydroponic culture; *Crop Sci.* **14** 790–794

Bonner J 1950 *Plant Biochemistry* (New York: Academic Press) 537 pp.

Coyne P I and Cook C W 1970 Seasonal Carbohydrate reserve cycles in eight desert range species; *J. Range Manage.* **23** 438–444

Davies A 1974 Leaf tissue remaining, after cutting and regrowth in perennial ryegrass; *J. Agric. Sci. Camb.* **82** 165–172

Detling J K, Dyer M I and Winn D 1979 Net photosynthesis, root respiration and regrowth of *Bouteloua*; *Oecologia* **41** 127–134

Evans G C 1972 *The Quantitative Analysis of Plant Growth.* (Oxford, London, Edinburgh, Melbourne: Blackwell Scientific Publications)

Gates C T and Bonner J 1959 The response of the young tomato plant to a brief period of water storage. IV. Effects of water stress on the ribonucleic acid metabolism of tomato leaves; *Plant Physiol* **34** 49–55

Higgs D E S and James D B 1969 Comparative studies on the biology of upland grasses. I. Rate of dry matter production and its control in four grasses; *J. Ecol.* **57** 533–563

Jarvis M S 1963 A comparison between the water relations of species with contrasting type of geographical distribution in British Isles; in *The Water Relations of Plants*. 289–312 eds. A J Rutter and P H Whiteheat (Oxford: Blackwell Scientific Publications)

Kleinendorst A and Brouwer R 1969 Growth responses of two clones of perennial ryegrass to excision of roots or shoots. Instituut Voor Biologisch en Schekunding onderzoek Van Landbouwgewassen, Wageningen, Jaarbock, 19–25

Loomis R S, Williams W A and Hall A D 1971 Agricultural Productivity; *Ann. Rev. Plant Physiol.* **22** 431–468

Mooney H A 1972 The carbon balance of plants; *Ann. Rev. Ecol. and Syst.* **3** 315–346

Owensby C E, Rains J R and McKendrick J D 1974 Effects of one year of intensive clipping on big bluestem; *J. Range Manage.* **27** 341–343

Painter E L and Detling J E 1981 Effects of defoliation on net photosynthesis and regrowth of western wheat grass; *J. Range Manage.* **34** 68–71

Pande H and Singh J S 1981 Comparative biomass and water status of four grass species grown under two soil water conditions; *J. Range Manage* **34** 480–484

Pande U and Singh J S 1980 A quantitative study of the forest floor, litter fall and nutrient return in an oak-conifer forest in Himalaya. I. Composition and dynamics of forest; *Acta Oecologia Oecologia Generalis* **2** 49–61

Piper C S 1944 *Soil and Plant Analysis* (New York: Interscience Publishers, Inc.)

Trlica M J Jr. and Cook C W 1971 Defoliation effects on carbohydrate reserves of desert species; *J. Range Manage* **24** 418–425

—— and Singh J S 1979 Translocation of assimilates and creation, distribution, utilization of reserves; in *Arid-land Ecosystem, Structure, Functioning and Management, Vol. I. IBP* eds. 16. R A Perry and D W Goodall (Camb. Uni. Press) 537–571

Willard E E and McKell C M 1978 Sprouting and Carbohydrate reserves of two wildland shrubs following partial defoliation; *J. Range Manage* **31** 2

Proc. Indian natn. Sci. Acad. B51 No. 5 pp 641–644 (1985)

PROF. TOPPUR SEETHAPATHY SADASIVAN MEMORIAL LECTURE, 1984

University Education in Environmental Science

R MISRA
Banaras Hindu University, Varanasi 221 005

(Delivered 1 August 1985)

It is a great pleasure and privilege to deliver Professor T S Sadasivan Endowment Lecture under the auspices of INSA-Varanasi Chapter. Professor Sadasivan is known for his deep insights into the ecology of host-parasite relationships. He was instrumental in initiating the University Grants Commission in 1971 for the institution of the Advanced Centre of Studies in Ecology at Botany Department of the Banaras Hindu University where I have taught and learnt the subject for about 40 years. Hence I am indebted to INSA and Professor D P Burma, the convener of the local Chapter respectively for awarding me the Lectureship and arranging the lecture at my 'alma mater'.

Few will disagree with the need of environmental education as aid for reorientation and support of our life style. Nevertheless, much deeper philosophical questions to which complete answers are not available at the moment, are involved in making the decision about the scope and content of such education. Our divergent views on the subject and intra- and inter-departmental rivalries are responsible for stalling action, especially at the University level. What kind of expertise, understanding and behaviour are expected of scholars and tomorrow's leaders in a society beset with consumerism leading to urbanisation and industrialisation in an era of ecological scarcity? An attempt is being made to meet this challenge through relevant and effective environmental education.

Society and environment are always in transition. Political, administrative and legal institutions are created to make the transition as smooth as possible. Education particularly in the Universities provides leadership for the purpose.

Environment is not only a matter of perception but an objective reality in which we are embedded for functioning. In turn the way we develop values and attitudes interacts, impacts and modifies the environment including ourselves to the extent of the experienced stresses. Thus environment is a web of perceived systems and factors which we can indicate, qualify and/or quantify for manipulating it to sustain a more spiritually and materially satisfying life. We use ecology as tool for understanding the environment as a system. Ecology is growing rapidly by internalising the different disciplines of natural and more recently social sciences. These other disciplines have also gained from ecology by applying many of its principles to their own discipline. Since ecology is rooted within the life sciences, interdisciplinary rivalries in the University system have made many scholars blind to its fertility in promoting a healthy growth of their own sciences. Indeed no discipline

has ever progressed without seeking information from other parts of knowledge as the latter is indivisible in the ultimate analysis.

When we encounter human environment and develop human ecology on the principles based on life sciences a reorientation of knowledge from all the sources is demanded. The skill for doing so makes ecology in the broadest sense like the skills we develop through education within each of the disciplines traditional to the University. These traditions have been zealously guarded according to predisposition of the scholars and the limitation of time devoted to training. Nevertheless every now and then Universities come up with proposals for instituting hybrid departments such as biochemistry, biophysics, econometrics, business management, etc. etc. thus breaking the barriers among the chosen disciplines.

Realising the importance of ecology and newer avenues of expanding knowledge traditional disciplines have incorporated in their studies environmentally oriented courses such as environmental biology, environmental physics, chemistry, geology, law, politics, engineering, etc. etc. In fact all the 120 teaching departments of the Banaras Hindu University interface with the environment in their respective fields. So a host of environmental courses are being administered. These activities though sectoral are highly desirable and as important as the schools of environmental sciences established at the Jawaharlal Nehru University, Andhra, Kerala (agriculture), Annamalai Universities etc. trying to understand the impact of specialised disciplines on the environment. Suitable degree and diploma courses as well as those related to inservice and public administration oriented courses are desinged for appreciation and reorientation in matters related to the environment and its enhancement. Nevertheless, they do not impart in-depth training in the management of ecosystems. Managers are required to forsee the impact of human activities on the environment from holistic approach and even advise specialists where they worsen the situation on account of their fragmentary knowledge and zeal for improving the environment. Very often well intentioned steps to reduce pollution, improve irrigation or drainage, plan human settlements, etc. etc. become counter-productive to the suitability of the whole environment because either the scale or the holistic approach of the ecologist has not been taken into account. Slogan shouting and agitations raised by the public are also focussed on political and so called ecological issues which are not examined in the context of the whole resulting into disproportioned growth spawning more and more difficult problems of the environment, since these are devoid of perspective ecological planning on a suitable scale of time and space. Hence, we need leaders and managers for environmental management. This task needs a thorough grounding in ecology and relating social trends with the way resources are used and consumed.

In view of the above discussion it is proposed to instal a full fledged two year M.Sc. course in environmental science which has to be erected as a single discipline training the mind to simulate environmental systems by transcending related traditional disciplines. Ecology has opened the door for such graduation. The subject oriented diploma and short term fragmentary courses are not relevant to either policy making or environmental designing for improving or maintaining the dynamic human environment as a whole.

Obviously environmental science as a single discipline cannot be the sum of all the environmental sciences including arts, physical and biological sciences, social sciences, etc. It ought to train the mind to establish linkages of interactions among them as observed in man's real world.

The real world can be observed as a set of phenomena in three distinct ways: (*i*) as discrete events, (*ii*) as sets of repetitive phenomena, similar individuals or populations or even aggregates of dissimilar populations, and (*iii*) as a set of interlinked processes or system. It is the systemic and holistic approach of ecology which differentiates it from other disciplines based on individuals and populations. Thus environmental education is training of the mind to grasp meaningful dynamics of the system of human societies and environments in transition as a whole. A person so trained can at once link up human activities with his surroundings extending right up to the biosphere. By quantifying the parameters one can develop a predictive model in which detailed processes can be telescoped or transcended according to the object in view such as impact analysis. A system model of any scale can be qualified, described in words or depicted in flow diagrams besides developing mathematical models.

The holistic study demands consideration of the functioning of the whole in relation to the subsystems within it. The meaning of the human environment thus unfolds itself within the context of a hierarchy of systems functioning within the biosphere and the universe. The functioning is achieved through the great biogeochemical cycles from which life has been evolving. The five basics of the cycle remain soil (earth), water, energy, space and air as conceived by the Indians from the Vedic time. The combinations, breakdown and recombinations of elements of these within space exhibit always newer phenomena not possessed by the combinants. The 'Brahamanda' or the Universe is the largest system containing all the real world phenomena. By subsuming the comprehensible systems as subsystems or ecosystems and with the aid of analytical and synthetic excercises we strike at man and his societies as evolving subsystems of the ecosystem of a given environment.

Man is a comparatively recent phenomenon of the biosphere. Besides his physical existence he is conscious of his perceptions of psychosocial, political and economic organizations, centered around the resources of living. He has also developed awareness of yet higher values of life such as spiritual, aesthetic and moral—very often subsumed within religion. These values interact and he expresses them in guiding his conduct through a hierarchy of value systems. Individuals, families and societies are guided by such orientation within the environment and so environment and society ever remain in transition. Thus environmental education has no meaning without an appreciation of the multidimensional man who has become the agent of change in both.

In the past era of ecological abundance science and technology propelled human institutions to exploit the resources of the biosphere. During the past two centuries industrial growth has exponentially accelerated the process. Now we are facing the stark reality of ecological scarcity. Nation states had differentiated during the period of ecological abundance. Those having precedence in resource exploitation have become disproportionately rich and so we have economic disorder leading to confrontation among the North and the South and the West and the East. Meanwhile ecological scarcity is compounded with two side growths of science and technology. These are nuclear power and communication explosion. So the world has become much smaller shivering with the prospect of nuclear holocaust and a much worse ecological scarcity with the denial of social justice. This challenge can be met only by a deeper appreciation, understanding and appropriate action through environmental education using all the means of communication.

It is naive not to recognise the pivotal role of environmental education for bringing about transformation in our political, legal

and administrative institutions designed to run smoothly societies right from village Panchayat to the United Nations organisations for the wise use of systems of environmental resources. We must realise that military threats to our survival are becoming more and more absurd. Never before was mankind capable of destroying itself not only as a possible result of the world-wide arms race but also as a result of the uncontrolled exploitation and destruction of the global resource base. The situation can be averted only by tempering our ego and greed through proper environmental education. It cannot be achieved by technological tinkering of the problems of pollution or settlements. We have to redesign a conservation oriented post-industrial society based on innovative and spiritual values.

In view of the scope and content of environmental education and reasons thereof given above the following suggestions are made for a proposed two year M.Sc. course:

Two Year M.Sc. Degree Syllabus

ENVIRONMENTAL SCIENCE

1. Comprehension of the holistic dimension of the environment and man's place in it. The ecosystem as a web of dynamic relationships.
2. Basic ecological principles. function, structure and evolution of populations and ecosystems. Driving and state variables of ecosystems. Limits to growth. Growth and Development. Density stresses.
3. Natural, man modified and built environments. Resources, their base and use systems. Wastes and pollution. Industrial growth.
4. Public and private enterprises and ecological scarcity. Governmental and social organisations. Global and international institutions concerned with resource management. Depletion of the environment. War and peace.
5. Competitive uses of land, water and air. Environmental problems. Their origin, intensification and sustainable solutions. Conservation, cooperation and coordination for resources use within ecosystems. Tragedy of the commons and the value of sacrifice and love for all beings.
6. Ethics, aesthetics, culture. Spiritual and moral values of life. Place of coercion and education. Value reorientation and environment. Mechanism of social changes.
7. Redesigning society and environment on variable scales. Modelling of ecosystems and simulation excercises.
8. Expectations from the post graduates in the environmental science. Their role in dissemination of knowledge and skill for environmental management. Public awareness, training and education both formal and non-formal about the state of the environment and planning.

Thanking you; I close.

Procedure for Submitting Manuscripts

General: Manuscripts should be sent *in triplicate* (with 3 sets of illustrations. *one* original and *two* photocopies) to the *Editor of Publications, Proceedings B, Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 110 002.*

Submission of the manuscript will be held to mply that it has not been previously published in any form and is not under consideration for publication elsewhere.

Presentation: Articles should be as brief as full documentation allows. They should not usually exceed 12 printed pages (approx. 12,000 words). Review articles may be up to 20 pages. Papers must be written clearly and concisely with consistency in style and spe llings (spellings should be according to Oxford Dictionary), and typed double spaced with ample margins on durable bond paper. The usual format is: Abstract, Key words, Introduction, Materials and Methods, Results, Discussion, Acknowledgement (if any) and References.

Title: Title should be brief, specific and informative of the subject discussed. It should not begin with such general words as 'The', 'A', 'Study', 'Effect'. If a paper forms part of a series, this may be indicated through a symbol in the title and a footnote "This is a paper X in series. Paper No. IX is ref.....". The preceding paper must then be included in the list of references.

Running Title: Should not exceed 50 characters (including spaces).

Key Words: Up to five important key words should be provided for indexing and for information retrieval.

Abbreviations: Non-standard abbreviations used five or more times in the text should be listed in the footnote. Define where first mentioned followed by abbreviations in parentheses. Subsequently, the abbreviations should be used. However, these should be kept to a minimum and should not be used in the titles, key words or abstract.

Abstract: The abstract (typed on a separate page) should summarize the principal findings within 250 words.

Text: The paper must be divided into sections preferably starting with 'Introduction' and ending with 'Discussion' (or 'Acknowledgement', if any). All measurements must be given in SI units. Avoid numbers at the beginning of a sentence or spell them out. The scientific names of plants and animals should be underlined. Authors of name of taxa should be cited at the first mention of a taxon, but not elsewhere. Accepted common names of plants and animals should neither be capitalized nor placed within quotation marks. Words and phrases of foreign origin in common use need not be underlined (e. g., et al., viz. i.e., etc.) whereas others should be underlined (e.g. *in vitro, in situ*).

Tables: All tables must be numbered serially in arabic numerals and placed at the end. The tables should have brief titles (*underlined*) and contents should be self-explanatory. Non-standard abbreviations should be used sparingly and defined at the bottom of the table. Also details of the experiment (not mentioned in the text) may be indicated below the table.

Illustrations: Original drawings (in ink in clean uniform lines on tracing paper or Bristol paper) and sharp photographs (with high contrast and glossy prints) numbered in arabic numerals should be provided. Illustrations on reduction must fit into *one-column* (6.5 cm) or *two-column* (13.5 cm) width. Labelling of figures should therefore be done keeping in view the reduction/ blow-up into one or two columns. Magnification should be indicated in the legend by scale or figures (×.....). For composite figures, mounting should be done carefully with even spacing in between. Illustrations should be supported with cardboard to avoid damage in transit.

References: References in the text should be cited by author and year, and not by number. If there are more than two authors, reference should be made to the first author followed by 'et al'. In the text. References at the end of the paper should be listed alphabetically by authors names. followed by initials, year of publication, title of the paper, name of the journal (underlined and abbreviated according to the 'World List of Scientific Periodicals, Butterworths, London') vol no. and page nos. References to books should include: Names of authors, initials, year of publication, title of the book (underlined), edition if not the first, initials and name(s) of editor(s), if any, preceded by 'eds', place of publication, publisher, and chapter or pages referred to. References to thesis must include the year, the title of the thesis, the degree for which submitted, the university and the place. Sample citations are given below :

Tata J R 1979 Control by estrogen of reversible gene expression: The vitellogenin model; *Proc. Indian natn. Sci. Acad.* B45 211-225

Warcup J H 1967 Fungi in soil; in *Soil Biology* (2nd edn) pp 1–16 eds A Burges and F Raw (London: Academic Press)

Proofs: Proofs will be sent to the author(s) and should be returned to the Editor *within 48 hours.* Major corrections or alterations will be accepted only at author's expense*. If proofs are not returned within the specified time, they will be read in the Editorial Office and print-ordered.

Reprints: Fifty reprints will be supplied free of charges to the authors. Additional copies should be ordered on the order-form accompanying the proofs. Payment for extra reprints must accompany the order-form.

---

*The recomposition cost will be charged to the author, in case the author desires to withdraw the paper after it has been set, he shall pay towards the composition charges.

CONTENTS (*Continued from outside back cover*)

Regd. No 16423/68

Proceedings of the Indian National Science Academy

Vol. 51, Part B, No. 5 October 1985

CONTENTS

(Continued on inside back cover)

Published by the Executive Secretary, Indian National Science Academy, Bahadur Shah Zafar Marg, New Delhi 110002 and Printed by him at The Central Electric Press, 80-D, Kamla Nagar, Delhi 110007